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(54) Title: USE OF 3,11B-CIS-DIHYDROTETRABENAZINE FOR THE TREATMENT OF SYMPTONS OF HUNTINGTON'S DISEASE

(57) Abstract: The invention provides 3,11 Ib-*cis*-dihydrotetrabenazine for use in halting or slowing the progress of one or more symptoms of Huntington's disease in a patient, and more particularly a symptom selected from involuntary movements such as involuntary chores, tremors and twitches, and degeneration in gait.

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USE OF 3,11B-CIS-DIHYDROTETRABENAZINE FOR THE TREATMENT OF SYMPTONS OF HUNTINGTON'S DISEASE

This invention relates to the use of dihydrotetrabenazine in treating Huntington's Disease.

Background of the Invention

Huntington's Disease, formerly known as Huntington's Chorea, is an inherited neuro-degenerative disease that is currently incurable. The disease is caused by a CAG trinucleotide repeat expansion (referred to as HD mutation) in the IT15 gene located on chromosome 4p16.3 which produces an abnormal form of a protein named Huntingtin. The abnormal protein triggers a process that results in the death of neurons in the corpus striatum region of the brain, possibly by the clumping or aggregation of the abnormal protein inside many types of neurons.

The Huntington's disease (HD) gene comprises a segment of DNA which contains the repeating sequence of nucleotides CAG coding for the amino acid glutamine. It has been found that if there are thirty or fewer CAG repeats within the gene, a person carrying the gene will not contract HD. However, persons carrying a gene in which there are over forty CAG repeats do tend to contract the disease.

Huntington's disease is transmitted via an autosomal dominant inheritance pattern such that each child of an HD-affected parent has a 50% chance of inheriting the disorder. The symptoms of Huntington's disease typically appear between the ages of about 30 and 50 years and the disease usually progresses over a 10 – 25 year period. The characteristics and symptoms of the disease include personality changes, depression, mood swings, unsteady gait, involuntary chorea, twitching and jerking movements and tremors, dementia, slurred speech, impaired judgement, difficulty in swallowing and an intoxicated appearance.

Once an individual becomes symptomatic for Huntington's disease the course of the disease can last anywhere from ten to thirty years. Typically, the course of HD can be roughly divided into three stages, the early, middle and late stages.

In the early stage, stage patients can still perform most of their usual activities. They may still be working and may still be able to drive. Whilst they may exhibit

slight uncontrollable movements, stumbling and clumsiness, lack of concentration, short-term memory lapses and depression, as well as mood swings, the involuntary movements are relatively mild, speech is still clear, and dementia, if present at all, is mild.

During the middle stage, patients become more disabled and typically need assistance with some of their routine daily activities. Falls, weight loss, and swallowing difficulties may be a problem during this stage and dementia becomes more obvious to the casual observer. In addition, the uncontrollable movements become more pronounced.

During the late stage, patients deteriorate to the point where they require almost total care and many require constant attention in hospitals or nursing homes. At this stage, they may no longer be able to walk or speak and, although they may show fewer involuntary movements, may become more rigid. Patients in this stage are often unable to swallow food. At this stage most patients lose insight and are apparently unaware of their surroundings. When the patient finally dies, the cause of death is usually related to the same natural causes that lead to death in other severely debilitated patients, such as malnutrition or pneumonia.

According to the US National Institute of Neurological Disorders and Stroke (NINDS), a part of the National Institute of Health (NIH), there is currently no way of stopping or reversing the course of Huntington's disease.

Attempts have been made to develop treatments for HD and one study by Karpuj *et al* in *Nature Medicine*, February 2002, vol.8, no.2, pp. 143 – 149 has involved the administration of cystamine. Apparently, the cystamine inactivates the enzyme transglutaminase which helps to create the clumps of Huntingtin protein thought to be responsible for the disease. Nevertheless, at present, so far as the applicants are aware, there is currently no generally available medicine for treating or arresting the progression of Huntington's disease.

The discovery of the gene responsible for Huntington's disease (see the paper by the Huntington's Disease Collaborative Group, *Cell*, Vol. 72, March 26, 1993, p. 971) has enabled diagnostic tests for the presence of the mutant form of the gene to be

developed. Diagnostic tests, which make use of the polymerase chain reaction (PCR) to detect the number of CAG repeats on the IT-15 gene, are now widely available and allow a prediction to be made whether or not a patient will develop the symptoms of Huntington's disease; see for example the review by M. Hayden *et al.*, *Am. J. Hum. Genet.* 55:606-617 (1994); the article by S. Hersch, "The Neurogenetics Genie: Testing for the Huntington's disease mutation." *Neurol.* 1994; 44:1369-1373; and the article by R. R. Brinkman *et al.* (1997) "The likelihood of being affected with Huntington disease by a particular age, for a specific CAG size", *Am. J. Hum. Genet.* 60:1202-1210.

Tetrabenazine (Chemical name: 1, 3, 4,6,7,11b-hexahydro-9,10-dimethoxy-3-(2-methylpropyl)-2H-benzo(a)quinolizin-2-one) has been in use as a pharmaceutical drug since the late 1950s. Initially developed as an anti-psychotic, tetrabenazine is currently used in the symptomatic treatment of hyperkinetic movement disorders such as Huntington's disease, hemiballismus, senile chorea, tic, tardive dyskinesia and Tourette's syndrome, see for example Jankovic *et al.*, *Am. J. Psychiatry.* (1999) Aug; 156(8):1279-81 and Jankovic *et al.*, *Neurology* (1997) Feb; 48(2):358-62.

The primary pharmacological action of tetrabenazine is to reduce the supply of monoamines (e.g. dopamine, serotonin, and norepinephrine) in the central nervous system by inhibiting the human vesicular monoamine transporter isoform 2 (hVMAT2). The drug also blocks postsynaptic dopamine receptors.

Tetrabenazine is an effective and safe drug for the treatment of a variety of hyperkinetic movement disorders and, in contrast to typical neuroleptics, has not been demonstrated to cause tardive dyskinesia. Nevertheless, tetrabenazine does exhibit a number of dose-related side effects including causing depression, Parkinsonism, drowsiness, nervousness or anxiety, insomnia and, in rare cases, neuroleptic malignant syndrome.

The central effects of tetrabenazine closely resemble those of reserpine, but it differs from reserpine in that it lacks activity at the VMAT1 transporter. The lack of activity at the VMAT1 transporter means that tetrabenazine has less peripheral activity than reserpine and consequently does not produce VMAT1-related side effects such as hypotension.

The chemical structure of tetrabenazine is as shown in Figure 1 below.

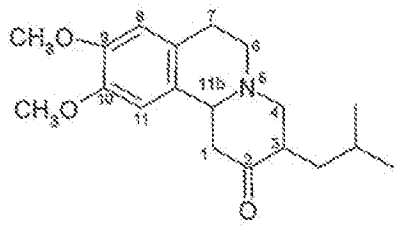


Figure 1- Structure of tetrabenazine

The compound has chiral centres at the 3 and 11b carbon atoms and hence can, theoretically, exist in a total of four isomeric forms, as shown in Figure 2.

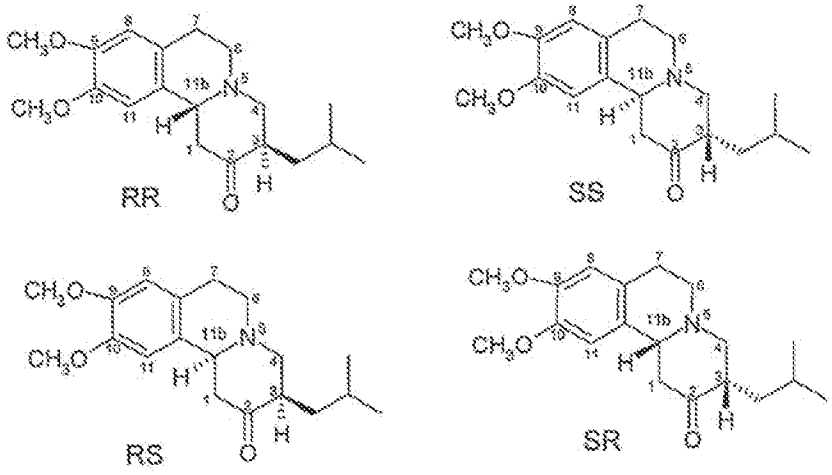


Figure 2 – Possible tetrabenazine isomers

In Figure 2, the stereochemistry of each isomer is defined using the "R and S" nomenclature developed by Cahn, Ingold and Prelog, see *Advanced Organic Chemistry* by Jerry March, 4th Edition, John Wiley & Sons, New York, 1992, pages 109-114. In Figure 2 and elsewhere in this patent application, the designations "R" or "S" are given in the order of the position numbers of the carbon atoms. Thus, for example, *RS* is a shorthand notation for 3*R*,11b*S*. Similarly, when three chiral centres are present, as in the dihydrotetrabenazines described below, the designations "*R*" or "*S*" are listed in the order of the carbon atoms 2, 3 and 11b. Thus, the 2*S*,3*R*,*11bR* isomer is referred to in short hand form as *SRR* and so on.

Commercially available tetrabenazine is a racemic mixture of the *RR* and *SS* isomers and it would appear that the *RR* and *SS* isomers (hereinafter referred to

individually or collectively as *trans*-tetrabenazine because the hydrogen atoms at the 3 and 11b positions have a *trans* relative orientation) are the most thermodynamically stable isomers.

Tetrabenazine has somewhat poor and variable bioavailability. It is extensively metabolised by first-pass metabolism, and little or no unchanged tetrabenazine is typically detected in the urine. The major metabolite is dihydrotetrabenazine (Chemical name: 2-hydroxy-3-(2-methylpropyl)-1,3,4,6,7,11b-hexahydro-9,10-dimethoxy-benzo(a)quinolizine) which is formed by reduction of the 2-keto group in tetrabenazine, and is believed to be primarily responsible for the activity of the drug (see Mehvar *et al.*, *Drug Metab.Disp*, 15, 250-255 (1987) and *J. Pharm. Sci.*, 76, No.6, 461-465 (1987)), and Roberts *et al.*, *Eur. J. Clin. Pharmacol.*, 29: 703-708 (1986).

Four dihydrotetrabenazine isomers have previously been identified and characterised, all of them being derived from the more stable *RR* and *SS* isomers of the parent tetrabenazine and having a *trans* relative orientation between the hydrogen atoms at the 3 and 11b positions) (see Kilbourn *et al.*, *Chirality*, 9:59-62 (1997) and Brossi *et al.*, *Helv. Chim. Acta.*, vol. XLI, No. 193, pp1793-1806 (1958). The four isomers are (+)-α-dihydrotetrabenazine, (-)-α-dihydrotetrabenazine, (+)-β-dihydrotetrabenazine and (-)-β-dihydrotetrabenazine. The structures of the four known dihydrotetrabenazine isomers are considered to be as shown in Figure 3.

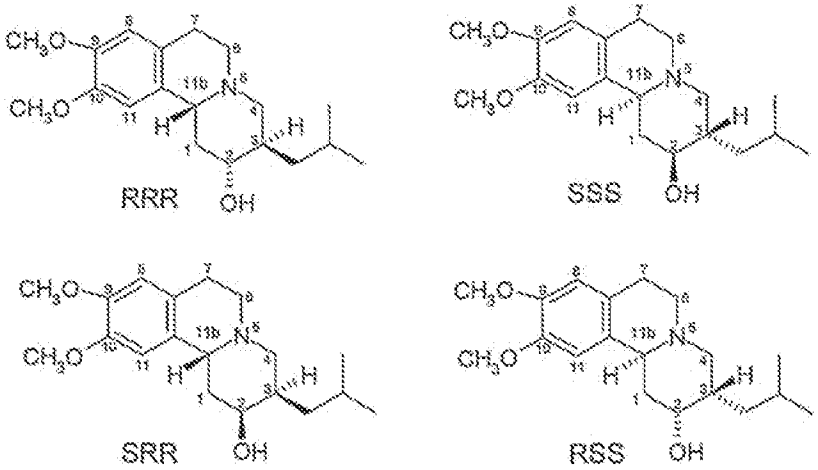


Figure 3 – Structures of known isomers of dihydrotetrabenazine

Kilbourn *et al.*,(see *Eur. J. Pharmacol.*, 278:249-252 (1995) and *Med. Chem. Res.*, 5:113-126 (1994)) investigated the specific binding of individual radio-labelled dihydrotetrabenazine isomers in the conscious rat brain. They found that the (+)-α-[^{11}C]dihydrotetrabenazine (2*R*,3*R*,11b*R*) isomer accumulated in regions of the brain associated with higher concentrations of the neuronal membrane dopamine transporter (DAT) and the vesicular monoamine transporter (VMAT2). However, the essentially inactive (-)-α-[^{11}C]dihydrotetrabenazine isomer was almost uniformly distributed in the brain, suggesting that specific binding to DAT and VMAT2 was not occurring. The *in vivo* studies correlated with *in vitro* studies which demonstrated that the (+)-α-[^{11}C]dihydrotetrabenazine isomer exhibits a K_i for [^{3}H]methoxytetrabenazine >2000-fold higher than the K_i for the (-)-α-[^{11}C]dihydrotetrabenazine isomer.

International patent application number PCT/GB2005/000464 (Publication number WO 2005/077946) discloses the preparation and use of pharmaceutical dihydrotetrabenazine isomers derived from the unstable *RS* and *SR* isomers (hereinafter referred to individually or collectively as *cis*-tetrabenazine because the hydrogen atoms at the 3 and 11b positions have a *cis* relative orientation) of tetrabenazine.

Summary of the Invention

It has now been found that the *cis*-dihydrotetrabenazine described in our earlier application no. PCT/GB2005/000464 has the ability to arrest or slow down the development of at least some of the symptoms of Huntington's disease. More particularly, it has been found that the deterioration of gait and the increase in involuntary movements (e.g. tremors and twitches) associated with Huntington's disease can be arrested or considerably slowed down by the administration of the *cis*-dihydrotetrabenazines of the invention.

Accordingly, in a first aspect, the invention provides 3, 11b-*cis*-dihydrotetrabenazine for use in halting or slowing the progress of one or more symptoms of Huntington's disease, and more particularly a symptom selected from involuntary movements such as involuntary chorea, tremors and twitches, and degeneration in gait.

In another aspect, the invention provides the use of 3, 11b-*cis*-dihydrotetrabenazine for the manufacture of a medicament for halting or slowing the progress of one or more symptoms of Huntington's disease, and more particularly a symptom selected from involuntary movements such as involuntary chorea, tremors and twitches, and gait degeneration.

In a still further aspect, the invention provides a method of halting or slowing the progress of one or more symptoms of Huntington's disease, and more particularly a symptom selected from involuntary movements such as involuntary chorea, tremors and twitches, and gait degeneration, in a patient in need of such treatment, which method comprises the administration of an effective therapeutic amount of 3,11b-*cis*-dihydrotetrabenazine.

Diagnostic tests are currently available for determining whether an individual is carrying the mutant Huntington's disease gene. Since a person carrying the mutant gene will almost invariably develop Huntington's disease, it would be advantageous if the onset or development of the disease could be prevented, arrested or slowed down during the period in a person's life at which he or she is most likely to develop the disease.

Accordingly, in a further aspect of the invention, there is provided a method for the prophylactic treatment of a patient identified as carrying the mutant gene responsible for Huntington's disease, the method comprising administering to the patient a *cis*-dihydrotetrabenazine as herein before defined in an amount effective to prevent or slow down the onset or progression of the disease.

In another aspect of the invention, there is provided a method for the prophylactic treatment of a patient identified as carrying the mutant gene responsible for Huntington's disease, the method comprising administering to the patient a *cis*-dihydrotetrabenazine as herein before defined in an amount effective to prevent or slow down sub-clinical progression of the disease. By sub-clinical progression is meant the development of the disease prior to the point at which the symptoms of the disease become apparent by clinical as opposed to biochemical investigation or investigation using scanning techniques such as computerised tomography or magnetic resonance imaging (MRI).

For example, the *cis*-dihydrotetrabenazine may be administered prophylactically to persons within the age range 15-50 years, e.g. 20 - 50 years or 25 - 50 years or 30 - 50 years, who are carrying the mutant form of the HD gene but who have not yet developed symptoms of the disease.

References to the mutant form of the Huntington's Disease gene or like expressions in this application refer to forms of the gene in which the number of CAG repeats on the IT-15 gene is at least thirty five, more typically at least forty, for example at least 45, or at least 50. In some cases, there may be a very high number of CAG repeats (e.g. 70 or above) and persons carrying a mutant form of the gene with such a large number of CAG repeats is likely to develop the juvenile-onset form of the disease.

In a further aspect therefore, the *cis*-dihydrotetrabenazine may be administered prophylactically to persons of less than 30 years in age, for example in the range 10-29, more typically 15-29 or 20-29 years in age who have been tested and have been found to have mutant forms of the IT-15 gene in which the number of CAG repeats exceeds 60, and more particularly is at least 65, and preferably is 70 or more.

The *cis*-dihydrotetrabenazine used in the present invention is 3, 11b, *cis*-dihydrotetrabenazine.

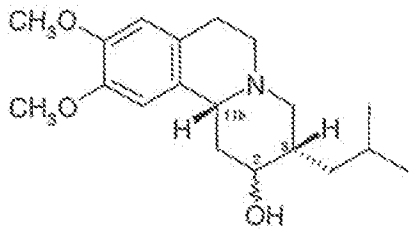
The 3,11b-*cis*-dihydrotetrabenazine used in the invention may be in substantially pure form, for example at an isomeric purity of greater than 90%, typically greater than 95% and more preferably greater than 98%.

The term "isomeric purity" in the present context refers to the amount of 3,11b-*cis*-dihydrotetrabenazine present relative to the total amount or concentration of dihydrotetrabenazine of all isomeric forms. For example, if 90% of the total dihydrotetrabenazine present in the composition is 3,11b-*cis*-dihydrotetrabenazine, then the isomeric purity is 90%.

The 11b-*cis*-dihydrotetrabenazine used in the invention may be in the form of a composition which is substantially free of 3,11b-*trans*-dihydrotetrabenazine,

preferably containing less than 5% of 3,11b-*trans*-dihydrotetrabenazine, more preferably less than 3% of 3,11b-*trans*-dihydrotetrabenazine, and most preferably less than 1% of 3,11b-*trans*-dihydrotetrabenazine.

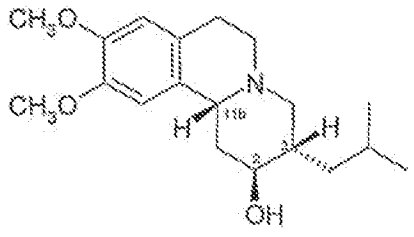
The term "3,11b-*cis*-" as used herein means that the hydrogen atoms at the 3- and 11b-positions of the dihydrotetrabenazine structure are in the *cis* relative orientation. The isomers of the invention are therefore compounds of the formula (I) and antipodes (mirror images) thereof.



(I)

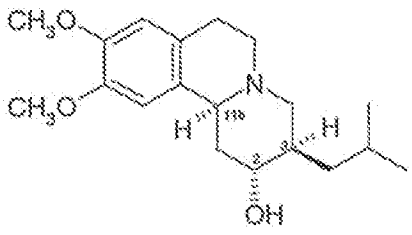
There are four possible isomers of dihydrotetrabenazine having the 3,11b-*cis* configuration and these are the 2*S*,3*S*,11b*R* isomer, the 2*R*,3*R*,11b*S* isomer, the 2*R*,3*S*,11b*R* isomer and the 2*S*,3*R*,11b*S* isomer. The four isomers have been isolated and characterised and, in another aspect, the invention provides individual isomers of 3,11b-*cis*-dihydrotetrabenazine. In particular, the invention provides:

(a) the 2*S*,3*S*,11b*R* isomer of 3,11b-*cis*-dihydrotetrabenazine having the formula (Ia):



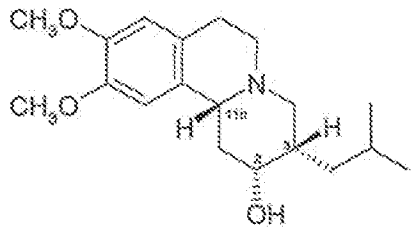
(Ia)

(b) the 2*R*,3*R*,11b*S* isomer of 3,11b-*cis*-dihydrotetrabenazine having the formula (Ib):



(Ib)

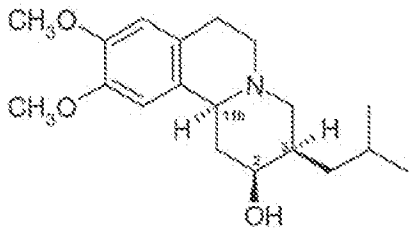
(c) the 2*R*,3*S*,11b*R* isomer of 3,11b-*cis*-dihydrotetrabenazine having the formula (Ic):



(Ic)

and

(d) the 2*S*,3*R*,11b*S* isomer of 3,11b-*cis*-dihydrotetrabenazine having the formula (Id):



The individual isomers of the invention can be characterised by their spectroscopic, optical and chromatographic properties, and also by their absolute stereochemical configurations as determined by X-ray crystallography.

Preferred isomers are the dextrorotatory (+) isomers.

A particularly preferred isomer is isomer (Ia), i.e. the 2*S*,3*S*,11b*R* isomer of 3,11b-*cis*-dihydrotetrabenazine.

Without implying any particular absolute configuration or stereochemistry, the four novel isomers may be characterised as follows:

Isomer A

Optical activity as measured by ORD (methanol, 21°C): laevorotatory (-)
IR Spectrum (KBr solid), ^{1}H-NMR spectrum ($CDCl_3$) and ^{13}C-NMR spectrum ($CDCl_3$) substantially as described in Table 1.

Isomer B

Optical activity as measured by ORD (methanol, 21°C): dextrorotatory (+)
IR Spectrum (KBr solid), ^{1}H-NMR spectrum ($CDCl_3$) and ^{13}C-NMR spectrum ($CDCl_3$) substantially as described in Table 1, and X-ray crystallographic properties as described in Example 4.

Isomer C

Optical activity as measured by ORD (methanol, 21°C): dextrorotatory (+)
IR Spectrum (KBr solid), ^{1}H-NMR spectrum ($CDCl_3$) and ^{13}C-NMR spectrum ($CDCl_3$) substantially as described in Table 2.

Isomer D

Optical activity as measured by ORD (methanol, 21°C): laevorotatory (-)
IR Spectrum (KBr solid), ^{1}H-NMR spectrum ($CDCl_3$) and ^{13}C-NMR spectrum ($CDCl_3$) substantially as described in Table 2.

ORD values for each isomer are given in the examples below but it is noted that such values are given by way of example and may vary according to the degree of purity of the isomer and the influence of other variables such as temperature fluctuations and the effects of residual solvent molecules.

The enantiomers A, B, C and D may each be presented in a substantially enantiomerically pure form or as mixtures with other enantiomers of the invention.

The terms "enantiomeric purity" and "enantiomerically pure" in the present context refer to the amount of a given enantiomer of 3,11b-*cis*-dihydrotetrabenazine present relative to the total amount or concentration of dihydrotetrabenazine of all enantiomeric and isomeric forms. For example, if 90% of the total dihydrotetrabenazine present in the composition is in the form of a single enantiomer, then the enantiomeric purity is 90%.

By way of example, in each aspect and embodiment of the invention, each individual enantiomer selected from Isomers A, B, C and D may be present in an enantiomeric purity of at least 55% (e.g. at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 99.5% or 100%).

The isomers of the invention may also be presented in the form of mixtures of one or more of Isomers A, B, C and D. Such mixtures may be racemic mixtures or non-racemic mixtures. Examples of racemic mixtures include the racemic mixture of Isomer A and Isomer B and the racemic mixture of Isomer C and Isomer D.

Pharmaceutically Acceptable Salts

Unless the context requires otherwise, a reference in this application to dihydrotetrabenazine and its isomers, includes within its scope not only the free base of the dihydrotetrabenazine but also its salts, and in particular acid addition salts.

Particular acids from which the acid addition salts are formed include acids having a pKa value of less than 3.5 and more usually less than 3. For example, the acid addition salts can be formed from an acid having a pKa in the range from +3.5 to -3.5.

Preferred acid addition salts include those formed with sulphonic acids such as methanesulphonic acid, ethanesulphonic acid, benzene sulphonic acid, toluene sulphonic acid, camphor sulphonic acid and naphthalene sulphonic acid.

One particular acid from which acid addition salts may be formed is methanesulphonic acid.

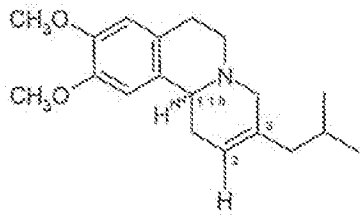
Acid addition salts can be prepared by the methods described herein or conventional chemical methods such as the methods described in *Pharmaceutical Salts: Properties, Selection, and Use*, P. Heinrich Stahl (Editor), Camille G. Wermuth (Editor), ISBN: 3-90639-026-8, Hardcover, 388 pages, August 2002. Generally, such salts can be prepared by reacting the free base form of the compound with the appropriate base or acid in water or in an organic solvent, or in

a mixture of the two; generally, nonaqueous media such as ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are used.

The salts are typically pharmaceutically acceptable salts. However, salts that are not pharmaceutically acceptable may also be prepared as intermediate forms which may then be converted into pharmaceutically acceptable salts. Such non-pharmaceutically acceptable salt forms also form part of the invention.

Methods for the Preparation of Dihydrotetrabenazine Isomers

The dihydrotetrabenazine of the invention can be prepared by a process comprising the reaction of a compound of the formula (II):



(II)

with a reagent or reagents suitable for hydrating the 2,3-double bond in the compound of formula (II) and thereafter where required separating and isolating a desired dihydrotetrabenazine isomer form.

The hydration of the 2,3-double bond can be carried out by hydroboration using a borane reagent such as diborane or a borane-ether (e.g. borane-tetrahydrofuran (THF)) to give an intermediate alkyl borane adduct followed by oxidation of the alkyl borane adduct and hydrolysis in the presence of a base. The hydroboration is typically carried out in a dry polar non-protic solvent such as an ether (e.g. THF), usually at a non-elevated temperature, for example room temperature. The borane-alkene adduct is typically oxidised with an oxidising agent such as hydrogen peroxide in the presence of a base providing a source of hydroxide ions, such as ammonium hydroxide or an alkali metal hydroxide, e.g. potassium hydroxide or sodium hydroxide. The hydroboration-oxidation-hydrolysis sequence of reactions of Process A typically provides dihydrotetrabenazine isomers in which the hydrogen atoms at the 2- and 3-positions have a *trans* relative orientation.

Compounds of the formula (II) can be prepared by reduction of tetrabenazine to give a dihydrotetrabenazine followed by dehydration of the dihydrotetrabenazine.

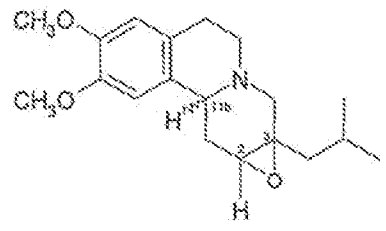
Reduction of the tetrabenazine can be accomplished using an aluminium hydride reagent such as lithium aluminium hydride, or a borohydride reagent such as sodium borohydride, potassium borohydride or a borohydride derivative, for example an alkyl borohydride such as lithium tri-*sec*-butyl borohydride. Alternatively, the reduction step can be effected using catalytic hydrogenation, for example over a Raney nickel or platinum oxide catalyst. Suitable conditions for performing the reduction step are described in more detail below or can be found in US 2,843,591 (Hoffmann- La Roche) and Brossi *et al.*, *Helv. Chim. Acta.*, vol. XLI, No. 193, pp1793-1806 (1958).

Because the tetrabenazine used as the starting material for the reduction reaction is typically a mixture of the *RR* and *SS* isomers (i.e. *trans*-tetrabenazine), the dihydrotetrabenazine formed by the reduction step will have the same *trans* configuration about the 3- and 11b positions and will take the form of one or more of the known dihydrotetrabenazine isomers shown in Figure 3 above. Thus Process A may involve taking the known isomers of dihydrotetrabenazine, dehydrating them to form the alkene (II) and then "rehydrating" the alkene (II) using conditions that give the required novel *cis* dihydrotetrabenazine isomers of the invention.

Dehydration of the dihydrotetrabenazine to the alkene (II) can be carried out using a variety of standard conditions for dehydrating alcohols to form alkenes, see for example J. March (*idem*) pages 389-390 and references therein. Examples of such conditions include the use of phosphorus-based dehydrating agents such as phosphorus halides or phosphorus oxyhalides, e.g. $POCl_3$ and PCl_5. As an alternative to direct dehydration, the hydroxyl group of the dihydrotetrabenazine can be converted to a leaving group L such as halogen (e.g. chlorine or bromine) and then subjected to conditions (e.g. the presence of a base) for eliminating H-L. Conversion of the hydroxyl group to a halide can be achieved using methods well known to the skilled chemist, for example by reaction with carbon tetrachloride or carbon tetrabromide in the presence of a trialkyl or triaryl phosphine such as triphenyl phosphine or tributyl phosphine.

The tetrabenazine used as the starting material for the reduction to give the dihydrotetrabenazine can be obtained commercially or can be synthesised by the method described in US 2,830,993 (Hoffmann-La Roche).

Another process (Process B) for preparing a dihydrotetrabenazine of the invention comprises subjecting a compound of the formula (III):



(III)

to conditions for ring-opening the 2,3-epoxide group in the compound of the formula (III), and thereafter where required separating and isolating a desired dihydrotetrabenazine isomer form.

The ring-opening can be effected in accordance with known methods for epoxide ring openings. However, a currently preferred method of ring-opening the epoxide is reductive ring opening which can be achieved using a reducing agent such as borane-THF. Reaction with borane-THF can be carried out in a polar non-protic solvent such as ether (e.g. tetrahydrofuran) usually at ambient temperature, the borane complex thus formed being subsequently hydrolysed by heating in the presence of water and a base at the reflux temperature of the solvent. Process B typically gives rise to dihydrotetrabenazine isomers in which the hydrogen atoms at the 2- and 3-positions have a *cis* relative orientation.

The epoxide compounds of the formula (III) can be prepared by epoxidation of an alkene of the formula (II) above. The epoxidation reaction can be carried out using conditions and reagents well known to the skilled chemist, see for example J. March (*idem*), pages 826-829 and references therein. Typically, a per-acid such as *meta*-chloroperbenzoic acid (MCPBA), or a mixture of a per-acid and a further oxidising agent such as perchloric acid, may be used to bring about epoxidation.

When the starting materials for processes A and B above are mixtures of enantiomers, then the products of the processes will typically be pairs of

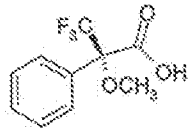
enantiomers, for example racemic mixtures, possibly together with diastereoisomeric impurities. Unwanted diastereoisomers can be removed by techniques such as chromatography (e.g. HPLC) and the individual enantiomers can be separated by a variety of methods known to the skilled chemist. For example, they can be separated by means of:

(i) chiral chromatography (chromatography on a chiral support); or

(ii) forming a salt with an optically pure chiral acid, separating the salts of the two diastereoisomers by fractional crystallisation and then releasing the dihydrotetrabenazine from the salt; or

(iii) forming a derivative (such as an ester) with an optically pure chiral derivatising agent (e.g. esterifying agent), separating the resulting epimers (e.g. by chromatography) and then converting the derivative to the dihydrotetrabenazine.

One method of separating pairs of enantiomers obtained from each of Processes A and B, and which has been found to be particularly effective, is to esterify the hydroxyl group of the dihydrotetrabenazine with an optically active form of Mosher's acid, such as the *R* (+) isomer shown below, or an active form thereof:



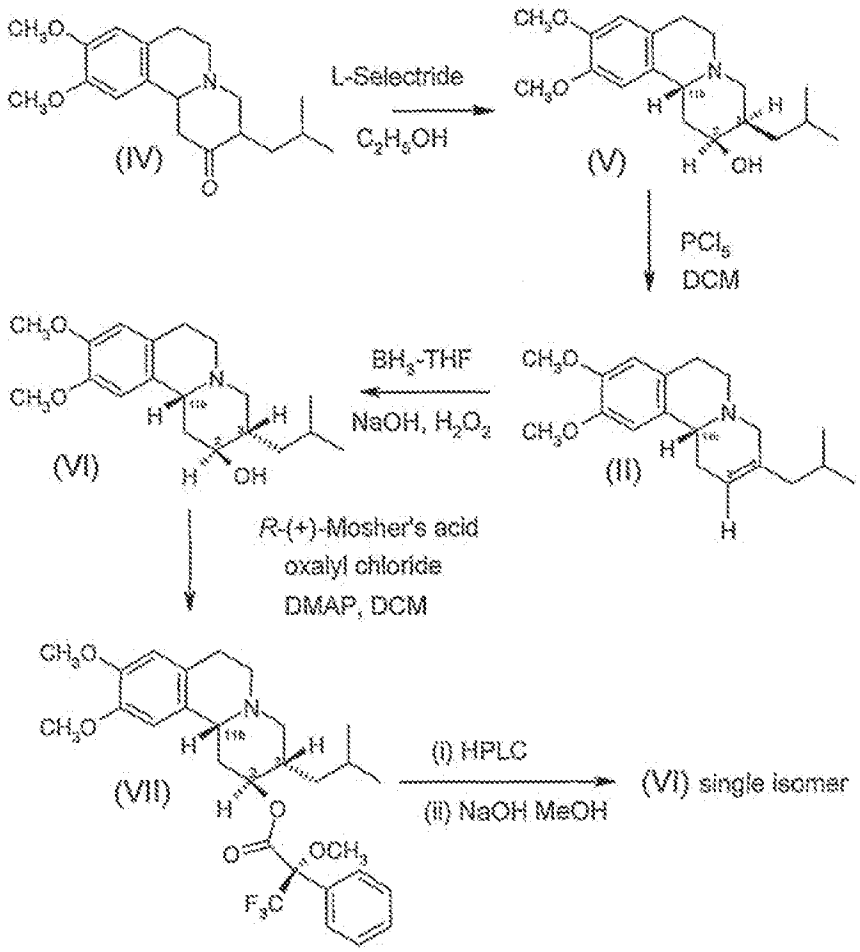
The resulting esters of the two enantiomers of the dihydrobenazine can then be separated by chromatography (e.g. HPLC) and the separated esters hydrolysed to give the individual dihydrobenazine isomers using a base such as an alkali metal hydroxide (e.g. NaOH) in a polar solvent such as methanol.

As an alternative to using mixtures of enantiomers as the starting materials in processes A and B and then carrying out separation of enantiomers subsequently, processes A and B can each be carried out on single enantiomer starting materials leading to products in which a single enantiomer predominates. Single enantiomers of the alkene (II) can be prepared by subjecting RR/SS tetrabenazine to a stereoselective reduction using lithium tri-*sec*-butyl borohydride to give a mixture of SRR and RSS enantiomers of dihydrotetrabenazine, separating the enantiomers (e.g. by fractional crystallisation) and then dehydrating a separated single

enantiomer of dihydrotetrabenazine to give predominantly or exclusively a single enantiomer of the compound of formula (II).

Processes A and B are illustrated in more detail below in Schemes 1 and 2 respectively.

Scheme 1



Scheme 1 illustrates the preparation of individual dihydrotetrabenazine isomers having the 2*S*,3*S*,11b*R* and 2*R*,3*R*,11b*S* configurations in which the hydrogen atoms attached to the 2- and 3-positions are arranged in a *trans* relative orientation. This reaction scheme includes Process A defined above.

The starting point for the sequence of reactions in Scheme 1 is commercially available tetrabenazine (IV) which is a racemic mixture of the RR and SS optical

isomers of tetrabenazine. In each of the RR and SS isomers, the hydrogen atoms at the 3- and 11b-positions are arranged in a *trans* relative orientation. As an alternative to using the commercially available compound, tetrabenazine can be synthesised according to the procedure described in US patent number 2,830,993 (see in particular example 11).

The racemic mixture of RR and SS tetrabenazine is reduced using the borohydride reducing agent lithium tri-*sec*-butyl borohydride ("L-Selectride") to give a mixture of the known 2*S*,3*R*,11b*R* and 2*R*,3*S*,11b*S* isomers (V) of dihydrotetrabenazine, of which only the 2*S*,3*R*,11b*R* isomer is shown for simplicity. By using the more sterically demanding L-Selectride as the borohydride reducing agent rather than sodium borohydride, formation of the RRR and SSS isomers of dihydro-tetrabenazine is minimised or suppressed.

The dihydrotetrabenazine isomers (V) are reacted with a dehydrating agent such as phosphorus pentachloride in a non-protic solvent such as a chlorinated hydrocarbon (for example chloroform or dichloromethane, preferably dichloromethane) to form the unsaturated compound (II) as a pair of enantiomers, only the *R*-enantiomer of which is shown in the Scheme. The dehydration reaction is typically carried out at a temperature lower than room temperature, for example at around 0-5°C.

The unsaturated compound (II) is then subjected to a stereoselective re-hydration to generate the dihydrotetrabenazine (VI) and its mirror image or antipode (not shown) in which the hydrogen atoms at the 3- and 11b-positions are arranged in a *cis* relative orientation and the hydrogen atoms at the 2- and 3-positions are arranged in a *trans* relative orientation. The stereoselective rehydration is accomplished by a hydroboration procedure using borane-THF in tetrahydrofuran (THF) to form an intermediate borane complex (not shown) which is then oxidised with hydrogen peroxide in the presence of a base such as sodium hydroxide.

An initial purification step may then be carried out (e.g. by HPLC) to give the product (V) of the rehydration reaction sequence as a mixture of the 2*S*,3*S*,11b*R* and 2*R*,3*R*,11b*S* isomers of which only the 2*S*,3*S*,11b*R* isomer is shown in the Scheme. In order to separate the isomers, the mixture is treated with *R* (+) Mosher's acid, in the presence of oxalyl chloride and dimethylaminopyridine

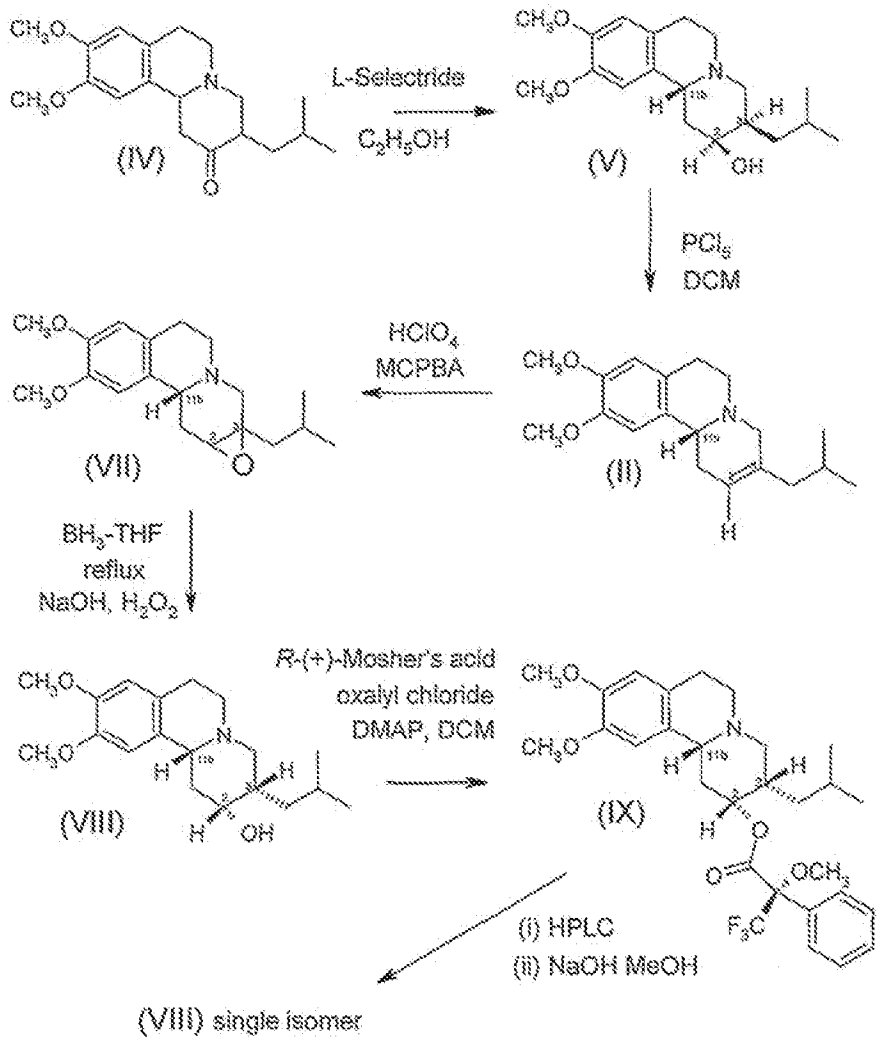
(DMAP) in dichloromethane to give a pair of diastereoisomeric esters (VII) (of which only one diastereoisomer is shown) which can then be separated using HPLC. The individual esters can then be hydrolysed using an alkali metal hydroxide such as sodium hydroxide to give a single isomer (VI).

In a variation of the sequence of steps shown in Scheme 1, following the reduction of RR/SS tetrabenazine, the resulting mixture of enantiomers of the dihydrotetrabenazine (V) can be separated to give the individual enantiomers. Separation can be carried out by forming a salt with a chiral acid such as (+) or (-) camphorsulphonic acid, separating the resulting diastereoisomers by fractional crystallisation to give a salt of a single enantiomer and then releasing the free base from the salt.

The separated dihydrotetrabenazine enantiomer can be dehydrated to give a single enantiomer of the alkene (II). Subsequent rehydration of the alkene (II) will then give predominantly or exclusively a single enantiomer of the cis-dihydrotetrabenazine (VI). An advantage of this variation is that it does not involve the formation of Mosher's acid esters and therefore avoids the chromatographic separation typically used to separate Mosher's acid esters.

Scheme 2 illustrates the preparation of individual dihydrotetrabenazine isomers having the 2*R*,3*S*,11b*R* and 2*S*,3*R*,11b*S* configurations in which the hydrogen atoms attached to the 2- and 3-positions are arranged in a *cis* relative orientation. This reaction scheme includes Process B defined above.

Scheme 2



In Scheme 2, the unsaturated compound (II) is produced by reducing tetrabenazine to give the 2S,3R,11bR and 2R,3S,11bS isomers (V) of dihydrotetrabenazine and dehydrating with PCl_5 in the manner described above in Scheme 1. However, instead of subjecting the compound (II) to hydroboration, the 2,3-double bond is converted to an epoxide by reaction with *meta*-chloroperbenzoic acid (MCPBA) and perchloric acid. The epoxidation reaction is conveniently carried out in an alcohol solvent such as methanol, typically at around room temperature.

The epoxide (VII) is then subjected to a reductive ring opening using borane-THF as an electrophilic reducing agent to give an intermediate borane complex (not shown) which is then oxidised and cleaved with hydrogen peroxide in the presence

of an alkali such as sodium hydroxide to give a dihydrotetrabenazine (VIII) as a mixture of the 2*R*,3*S*,11b*R* and 2*S*,3*R*,11b*S* isomers, of which only the 2*R*,3*S*,11b*R* is shown for simplicity. Treatment of the mixture of isomers (VIII) with *R* (+) Mosher's acid in the presence of oxalyl chloride and dimethylaminopyridine (DMAP) in dichloromethane gives a pair of epimeric esters (IX) (of which only one epimer is shown) which can then by separated by chromatography and hydrolysed with sodium hydroxide in methanol in the manner described above in relation to Scheme 1.

Biological Properties and Therapeutic Uses

Tetrabenazine exerts its therapeutic effects by inhibiting the vesicular monoamine transporter VMAT2 in the brain and by inhibiting both pre-synaptic and post-synaptic dopamine receptors.

The novel dihydrotetrabenazine isomers of the invention are also inhibitors of VMAT2, with Isomers C and B producing the greatest degree of inhibition. Like tetrabenazine, the compounds of the invention have only a low affinity for VMAT1, the VMAT isoform found in peripheral tissues and some endocrine cells, thereby indicating that they should not produce the side effects associated with reserpine. Compounds C and B also exhibit no inhibitory activity against catechol O-methyl transferase (COMT), monoamine oxidase isoforms A and B, and 5-hydroxytryptamine isoforms 1d and 1b.

Surprisingly, isomers C and B also show a remarkable separation of VAMT2 and dopamine receptor activity in that although they are highly active in binding VMAT2, both compounds exhibit only weak or non-existent dopamine receptor binding activity and lack Dopamine Transporter (DAT) binding activity. In fact, none of the isomers exhibit significant DAT binding activity. This suggests that the compounds may lack the dopaminergic side effects produced by tetrabenazine. Isomers C and B are also either weakly active or inactive as inhibitors of the adrenergic receptors and this suggests that the compounds may lack the adrenergic side effects often encountered with tetrabenazine. In fact, in locomotor studies carried out on rats, tetrabenazine exhibited a dose related sedative effect, whereas

no sedative effects were observed following administration of the dihydrotetrabenazine isomers B and C of the invention.

Furthermore, both Isomer C and Isomer B are potent inhibitors of the serotonin transporter protein SERT. Inhibition of SERT is one mechanism by which antidepressants such as fluoxetine (Prozac®) exert their therapeutic effects. Therefore, the ability of Isomers C and B to inhibit SERT indicates that these isomers may act as antidepressants, in marked contrast to tetrabenazine for which depression is a well recognised side effect.

Isomer B has been tested in a transgenic mouse model of Huntington's disease and has been shown to arrest the progression of a number of symptoms of Huntington's disease, including involuntary movements such as involuntary chorea, tremors and twitches, and deterioration in gait. On the basis of the studies carried out to date, it is envisaged that the *cis*-dihydrotetrabenazine compounds of the invention will therefore be useful in the treatment of Huntington's disease, and in particular for arresting or slowing down the progression of the disease, or for use in a prophylactic manner to prevent development of the disease.

The compounds will generally be administered to a subject in need of such administration, for example a human or animal patient, preferably a human.

The compounds will typically be administered in amounts that are therapeutically or prophylactically useful and which generally are non-toxic. However, in certain situations, the benefits of administering a dihydrotetrabenazine compound of the invention may outweigh the disadvantages of any toxic effects or side effects, in which case it may be considered desirable to administer compounds in amounts that are associated with a degree of toxicity.

A typical daily dose of the compound can be up to 1000 mg per day, for example in the range from 0.01 milligrams to 10 milligrams per kilogram of body weight, more usually from 0.025 milligrams to 5 milligrams per kilogram of body weight, for example up to 3 milligrams per kilogram of bodyweight, and more typically 0.15 milligrams to 5 milligrams per kilogram of bodyweight although higher or lower doses may be administered where required.

By way of example, an initial starting dose of 12.5 mg may be administered 2 to 3 times a day. The dosage can be increased by 12.5 mg a day every 3 to 5 days until the maximal tolerated and effective dose is reached for the individual as determined by the physician. Ultimately, the quantity of compound administered will be commensurate with the nature of the disease or physiological condition being treated and the therapeutic benefits and the presence or absence of side effects produced by a given dosage regimen, and will be at the discretion of the physician.

Pharmaceutical Formulations

The dihydrotetrabenazine compounds are typically administered in the form of pharmaceutical compositions.

The pharmaceutical compositions can be in any form suitable for oral, parenteral, topical, intranasal, intrabronchial, ophthalmic, otic, rectal, intra-vaginal, or transdermal administration. Where the compositions are intended for parenteral administration, they can be formulated for intravenous, intramuscular, intraperitoneal, subcutaneous administration or for direct delivery into a target organ or tissue by injection, infusion or other means of delivery.

Pharmaceutical dosage forms suitable for oral administration include tablets, capsules, caplets, pills, lozenges, syrups, solutions, sprays, powders, granules, elixirs and suspensions, sublingual tablets, sprays, wafers or patches and buccal patches.

Pharmaceutical compositions containing the dihydrotetrabenazine compounds of the invention can be formulated in accordance with known techniques, see for example, Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA, USA.

Thus, tablet compositions can contain a unit dosage of active compound together with an inert diluent or carrier such as a sugar or sugar alcohol, e.g.; lactose, sucrose, sorbitol or mannitol; and/or a non-sugar derived diluent such as sodium carbonate, calcium phosphate, talc, calcium carbonate, or a cellulose or derivative thereof such as methyl cellulose, ethyl cellulose, hydroxypropyl methyl cellulose,

and starches such as corn starch. Tablets may also contain such standard ingredients as binding and granulating agents such as polyvinylpyrrolidone, disintegrants (e.g. swellable crosslinked polymers such as crosslinked carboxymethylcellulose), lubricating agents (e.g. stearates), preservatives (e.g. parabens), antioxidants (e.g. BHT), buffering agents (for example phosphate or citrate buffers), and effervescent agents such as citrate/bicarbonate mixtures. Such excipients are well known and do not need to be discussed in detail here.

Capsule formulations may be of the hard gelatin or soft gelatin variety and can contain the active component in solid, semi-solid, or liquid form. Gelatin capsules can be formed from animal gelatin or synthetic or plant derived equivalents thereof.

The solid dosage forms (e.g.; tablets, capsules etc.) can be coated or un-coated, but typically have a coating, for example a protective film coating (e.g. a wax or varnish) or a release controlling coating. The coating (e.g. a Eudragit ™ type polymer) can be designed to release the active component at a desired location within the gastro-intestinal tract. Thus, the coating can be selected so as to degrade under certain pH conditions within the gastrointestinal tract, thereby selectively release the compound in the stomach or in the ileum or duodenum.

Instead of, or in addition to, a coating, the drug can be presented in a solid matrix comprising a release controlling agent, for example a release delaying agent which may be adapted to selectively release the compound under conditions of varying acidity or alkalinity in the gastrointestinal tract. Alternatively, the matrix material or release retarding coating can take the form of an erodible polymer (e.g. a maleic anhydride polymer) which is substantially continuously eroded as the dosage form passes through the gastrointestinal tract.

Compositions for topical use include ointments, creams, sprays, patches, gels, liquid drops and inserts (for example intraocular inserts). Such compositions can be formulated in accordance with known methods.

Compositions for parenteral administration are typically presented as sterile aqueous or oily solutions or fine suspensions, or may be provided in finely divided

sterile powder form for making up extemporaneously with sterile water for injection.

Examples of formulations for rectal or intra-vaginal administration include pessaries and suppositories which may be, for example, formed from a shaped mouldable or waxy material containing the active compound.

Compositions for administration by inhalation may take the form of inhalable powder compositions or liquid or powder sprays, and can be administrated in standard form using powder inhaler devices or aerosol dispensing devices. Such devices are well known. For administration by inhalation, the powdered formulations typically comprise the active compound together with an inert solid powdered diluent such as lactose.

The compounds of the inventions will generally be presented in unit dosage form and, as such, will typically contain sufficient compound to provide a desired level of biological activity. For example, a formulation intended for oral administration may contain from 2 milligrams to 200 milligrams of active ingredient, more usually from 10 milligrams to 100 milligrams, for example, 12.5 milligrams, 25 milligrams and 50 milligrams.

The active compound will be administered to a patient in need thereof (for example a human or animal patient) in an amount sufficient to achieve the desired therapeutic effect.

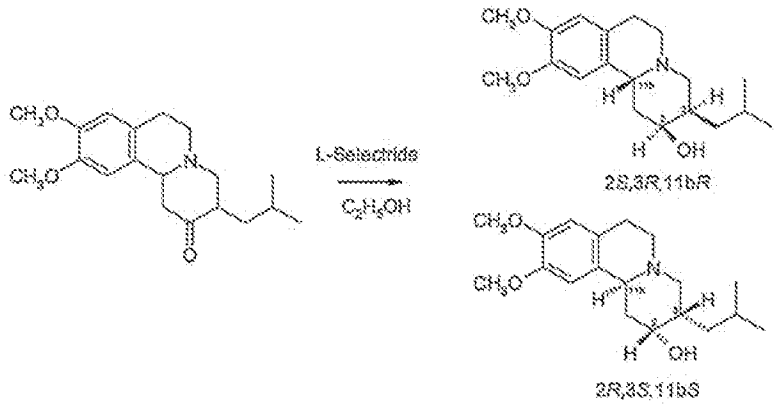
EXAMPLES

The following non-limiting examples illustrate the synthesis and properties of the dihydrotetrabenazine compounds of the invention.

EXAMPLE 1

Preparation of 2*S*,3*S*,11b*R* and 2*R*,3*R*,11b*S* Isomers of Dihydrotetrabenazine

1A. Reduction of *RR/SS* Tetrabenazine

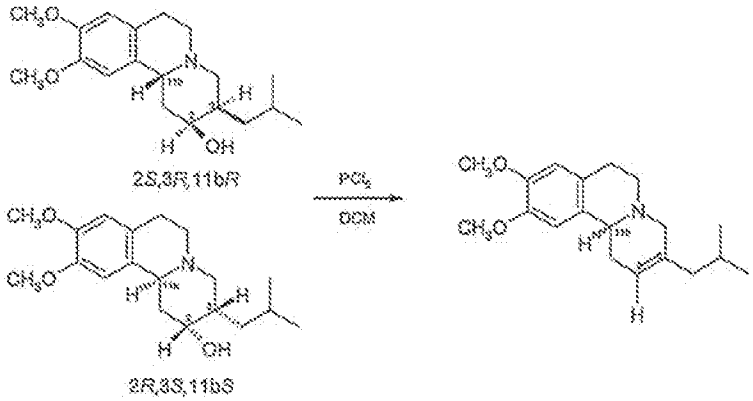


1M L-Selectride® in tetrahydrofuran (135 ml, 135 mmol, 2.87 eq.) was added slowly over 30 minutes to a stirred solution of tetrabenazine *RR/SS* racemate (15 g, 47 mmol) in ethanol (75 ml) and tetrahydrofuran (75 ml) at 0 °C. After addition was complete the mixture was stirred at 0 °C for 30 minutes and then allowed to warm to room temperature.

The mixture was poured onto crushed ice (300 g) and water (100 ml) added. The solution was extracted with diethyl ether (2 x 200 ml) and the combined ethereal extracts washed with water (100 ml) and partly dried over anhydrous potassium carbonate. Drying was completed using anhydrous magnesium sulphate and, after filtration, the solvent was removed at reduced pressure (shielded from the light, bath temperature <20 °C) to afford a pale yellow solid.

The solid was slurried with petroleum ether (30-40 °C) and filtered to afford a white powdery solid (12 g, 80%).

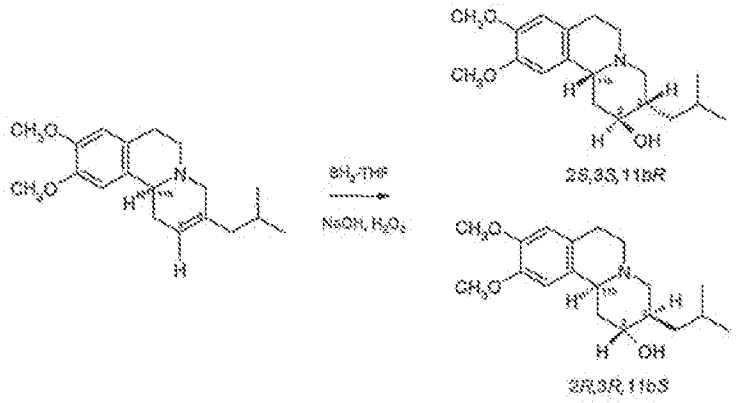
1B. Dehydration of reduced Tetrabenazine



Phosphorous pentachloride (32.8 g, 157.5 mmol, 2.5 eq) was added in portions over 30 minutes to a stirred solution of the reduced tetrabenazine product from Example 1A (20 g, 62.7 mmol) in dichloromethane (200 ml) at 0 °C. After the addition was complete, the reaction mixture was stirred at 0 °C for a further 30 minutes and the solution poured slowly into 2M aqueous sodium carbonate solution containing crushed ice (0 °C). Once the initial acid gas evolution had ceased the mixture was basified (ca. pH 12) using solid sodium carbonate.

The alkaline solution was extracted using ethyl acetate (800 ml) and the combined organic extracts dried over anhydrous magnesium sulphate. After filtration the solvent was removed at reduced pressure to afford a brown oil, which was purified by column chromatography (silica, ethyl acetate) to afford the semi-pure alkene as a yellow solid (10.87 g, 58%).

1C. Hydration of the Crude Alkene from Example 1B



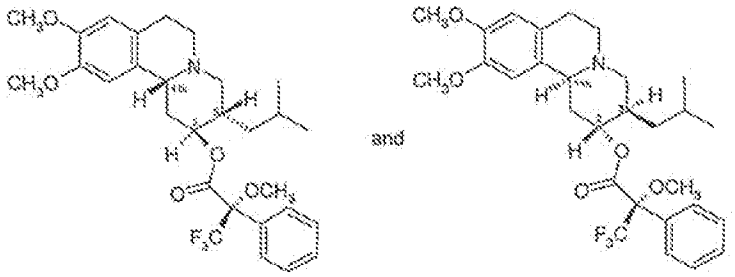
A solution of the crude alkene (10.87 g, 36.11 mmol) from Example 1B in dry THF (52 ml) at room temperature was treated with 1M borane-THF (155.6 ml, 155.6 mmol, 4.30 eq) added in a dropwise manner. The reaction was stirred for 2 hours, water (20 ml) was added and the solution basified to pH 12 with 30% aqueous sodium hydroxide solution.

Aqueous 30% hydrogen peroxide solution (30 ml) was added to the stirred alkaline reaction mixture and the solution was heated to reflux for 1 hour before being allowed to cool. Water (100 ml) was added and the mixture extracted with ethyl acetate (3 x 250 ml). The organic extracts were combined and dried over

anhydrous magnesium sulphate and after filtration the solvent was removed at reduced pressure to afford a yellow oil (9 g).

The oil was purified using preparative HPLC (Column: Lichrospher Si60, 5 μm, 250 x 21.20 mm, mobile phase: hexane : ethanol : dichloromethane (85:15:5); UV 254 nm, flow: 10 ml min^{-1}) at 350 mg per injection followed by concentration of the fractions of interest under vacuum. The product oil was then dissolved in ether and concentrated once more under vacuum to give the dihydrotetrabenazine racemate shown above as a yellow foam (5.76 g, 50%).

1D. Preparation of Mosher's ester derivatives



R-(+)-α-methoxy-α-trifluoromethylphenyl acetic acid (5 g, 21.35 mmol), oxalyl chloride (2.02 ml) and DMF (0.16 ml) were added to anhydrous dichloromethane (50 ml) and the solution was stirred at room temperature for 45 minutes. The solution was concentrated under reduced pressure and the residue was taken up in anhydrous dichloromethane (50 ml) once more. The resulting solution was cooled using an ice-water bath and dimethylaminopyridine (3.83 g, 31.34 mmol) was added followed by a pre-dried solution (over 4Å sieves) in anhydrous dichloromethane of the solid product of Example 1C (5 g, 15.6 mmol). After stirring at room temperature for 45 minutes, water (234 ml) was added and the mixture extracted with ether (2 x 200 ml). The ether extract was dried over anhydrous magnesium sulphate, passed through a pad of silica and the product eluted using ether.

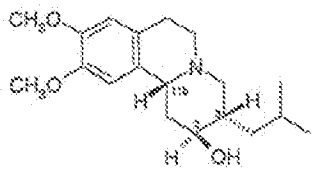
The collected ether eluate was concentrated under reduced pressure to afford an oil which was purified using column chromatography (silica, hexane : ether (10:1)). Evaporation of the collected column fractions of interest and removal of the solvent

at reduced pressure gave a solid which was further purified using column chromatography (silica, hexane : ethyl acetate (1:1)) to give three main components which were partially resolved into Mosher's ester peaks 1 and 2.

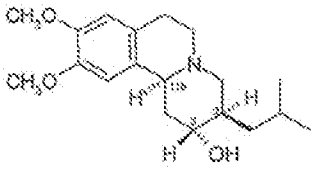
Preparative HPLC of the three components (Column: 2 x Lichrospher Si60, 5 μm, 250 x 21.20 mm, mobile phase: hexane : isopropanol (97:3), UV 254 nm; flow: 10 ml min^{-1}) at 300 mg loading followed by concentration of the fractions of interest under vacuum gave the pure Mosher's ester derivatives

Peak 1 (3.89 g, 46.5%)
Peak 2 (2.78 g, 33%)

The fractions corresponding to the two peaks were subjected to hydrolysis to liberate the individual dihydrotetrabenazine isomers identified and characterised as Isomers A and B. Isomers A and B are each believed to have one of the following structures



2*S*,3*S*,11b*R*



2*R*,3*R*,11b*S*

More specifically, Isomer B is believed to have the 2*S*, 3*S*, 11b*R* absolute configuration on the basis of the X-ray crystallography experiments described in Example 4 below.

1E. Hydrolysis of Peak 1 to give Isomer A

Aqueous 20% sodium hydroxide solution (87.5 ml) was added to a solution of Mosher's ester peak 1 (3.89 g, 7.27 mmol) in methanol (260 ml) and the mixture stirred and heated to reflux for 150 minutes. After cooling to room temperature water (200 ml) was added and the solution extracted with ether (600 ml), dried over anhydrous magnesium sulphate and after filtration, concentrated under reduced pressure.

The residue was dissolved using ethyl acetate (200 ml), the solution washed with water (2 x 50 ml), the organic phase dried over anhydrous magnesium sulphate and after filtration, concentrated under reduced pressure to give a yellow foam. This material was purified by column chromatography (silica, gradient elution of ethyl acetate : hexane (1:1) to ethyl acetate). The fractions of interest were combined and the solvent removed at reduced pressure. The residue was taken up in ether and the solvent removed at reduced pressure once more to give Isomer A as an off-white foam (1.1 g, 47%).

Isomer A, which is believed to have the 2*R*,3*R*,11b*S* configuration (the absolute stereochemistry was not determined), was characterized by ^{1}H-NMR, ^{13}C-NMR, IR, mass spectrometry, chiral HPLC and ORD. The IR, NMR and MS data for isomer A are set out in Table 1 and the Chiral HPLC and ORD data are set out in Table 3.

1F. Hydrolysis of Peak 2 to give Isomer B

Aqueous 20% sodium hydroxide solution (62.5 ml) was added to a solution of Mosher's ester peak 2 (2.78 g, 5.19 mmol) in methanol (185 ml) and the mixture stirred and heated to reflux for 150 minutes. After cooling to room temperature water (142 ml) was added and the solution extracted with ether (440 ml), dried over anhydrous magnesium sulphate and after filtration, concentrated under reduced pressure.

The residue was dissolved using ethyl acetate (200 ml), the solution washed with water (2 x 50 ml), the organic phase dried over anhydrous magnesium sulphate and after filtration, concentrated under reduced pressure. Petroleum ether (30-40 °C) was added to the residue and the solution concentrated under vacuum once more to give Isomer B as a white foam (1.34 g, 81%).

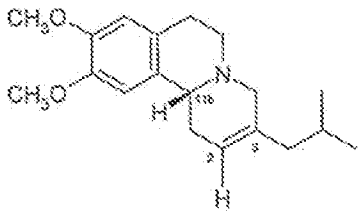
Isomer B, which is believed to have the 2*S*,3*S*,11b*R* configuration, was characterized by ^{1}H-NMR, ^{13}C-NMR, IR, mass spectrometry, chiral HPLC, ORD and X-ray crystallography. The IR, NMR and MS data for Isomer B are set out in Table 1 and the Chiral HPLC and ORD data are set out in Table 3. The X-ray crystallography data are set out in Example 4.

EXAMPLE 2

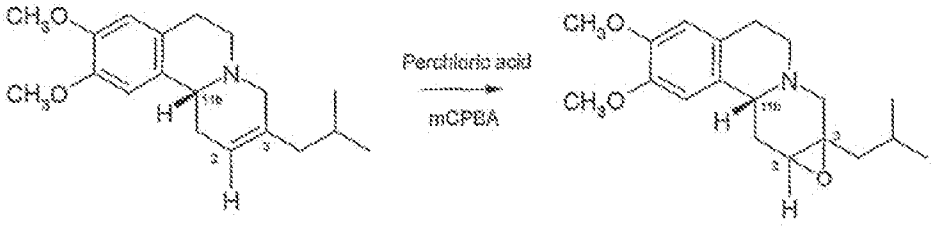
Preparation of 2*R*,3*S*,11b*R* and 2*S*,3*R*,11b*S* Isomers of Dihydrotetrabenazine

2A. Preparation of 2,3-Dehydrotetrabenazine

A solution containing a racemic mixture (15 g, 47 mmol) of *RR* and *SS* tetrabenazine enantiomers in tetrahydrofuran was subjected to reduction with L-Selectride® by the method of Example 1A to give a mixture of the 2*S*,3*R*,11b*R* and 2*R*,3*S*,11b*S* enantiomers of dihydrotetrabenazine.as a white powdery solid (12 g, 80%). The partially purified dihydrotetrabenazine was then dehydrated using PCl_5 according to the method of Example 1B to give a semi-pure mixture of 11b*R* and 11b*S* isomers of 2,3-dehydrotetrabenazine (the 11b*R* enantiomer of which is shown below) as a yellow solid (12.92 g, 68%).



2B. Epoxidation of the Crude Alkene from Example 2A



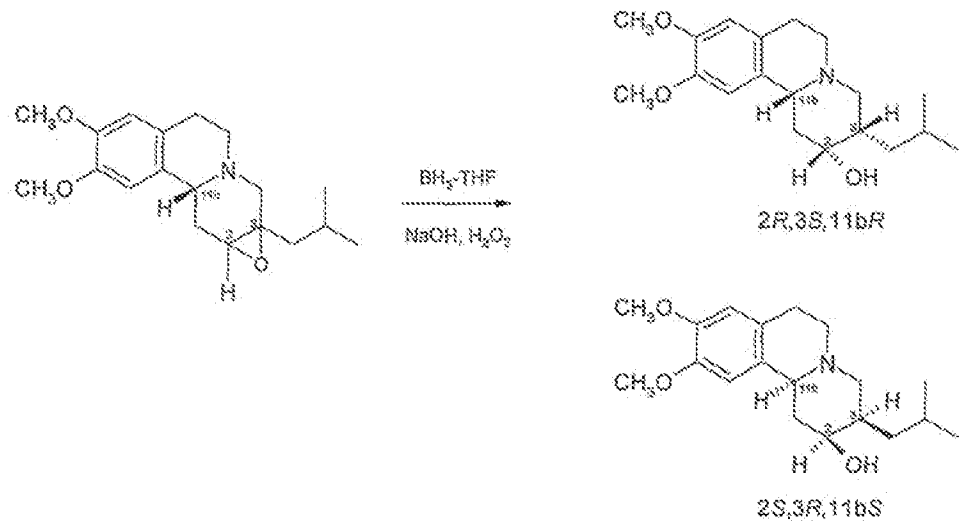
To a stirred solution of the crude alkene from Example 2A (12,92 g, 42.9 mmol) in methanol (215 ml) was added a solution of 70% perchloric acid (3.70 ml, 43 mmol) in methanol (215 ml). 77% 3-Chloroperoxybenzoic acid (15.50 g, 65 mmol) was added to the reaction and the resulting mixture was stirred for 18 hours at room temperature protected from light.

The reaction mixture was poured into saturated aqueous sodium sulphite solution (200 ml) and water (200 ml) added. Chloroform (300 ml) was added to the

resulting emulsion and the mixture basified with saturated aqueous sodium bicarbonate (400 ml).

The organic layer was collected and the aqueous phase washed with additional chloroform (2 x 150 ml). The combined chloroform layers were dried over anhydrous magnesium sulphate and after filtration the solvent was removed at reduced pressure to give a brown oil (14.35 g, yield > 100% - probable solvent remains in product). This material was used without further purification.

2C. Reductive Ring Opening of the Epoxide from 2B



A stirred solution of the crude epoxide from Example 2B (14.35 g, 42.9 mmol, assuming 100% yield) in dry THF (80 ml) was treated slowly with 1M borane/THF (184.6 ml, 184.6 mmol) over 15 minutes. The reaction was stirred for two hours, water (65 ml) was added and the solution heated with stirring to reflux for 30 minutes.

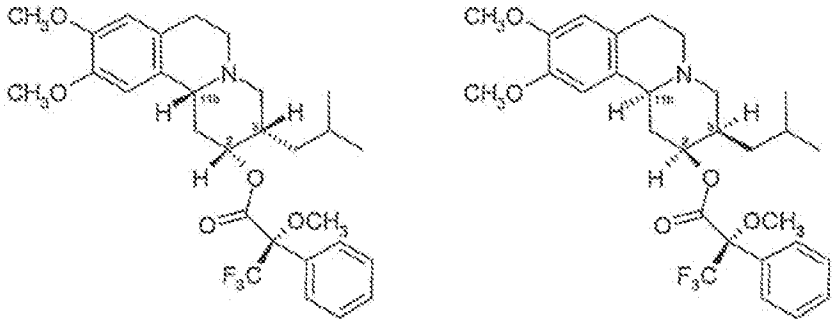
After cooling, 30% sodium hydroxide solution (97 ml) was added to the reaction mixture followed by 30% hydrogen peroxide solution (48.6 ml) and the reaction was stirred and heated to reflux for an additional 1 hour.

The cooled reaction mixture was extracted with ethyl acetate (500 ml) dried over anhydrous magnesium sulphate and after filtration the solvent was removed at

reduced pressure to give an oil. Hexane (230 ml) was added to the oil and the solution re-concentrated under reduced pressure.

The oily residue was purified by column chromatography (silica, ethyl acetate). The fractions of interest were combined and the solvent removed under reduced pressure. The residue was purified once more using column chromatography (silica, gradient, hexane to ether). The fractions of interest were combined and the solvents evaporated at reduced pressure to give a pale yellow solid (5.18 g, 38%).

2D. Preparation of Mosher's ester derivatives of the 2*R*,3*S*,11b*R* and 2*S*,3*R*,11b*S* Isomers of Dihydrotetrabenazine



R-(+)-α-methoxy-α-trifluoromethylphenyl acetic acid (4.68 g, 19.98 mmol), oxalyl chloride (1.90 ml) and DMF (0.13 ml) were added to anhydrous dichloromethane (46 ml) and the solution stirred at room temperature for 45 minutes. The solution was concentrated under reduced pressure and the residue was taken up in anhydrous dichloromethane (40 ml) once more. The resulting solution was cooled using an ice-water bath and dimethylaminopyridine (3.65 g, 29.87 mmol) was added followed by a pre-dried solution (over 4Å sieves) in anhydrous dichloromethane (20 ml) of the solid product of Example 2C (4.68 g, 14.6 mmol). After stirring at room temperature for 45 minutes, water (234 ml) was added and the mixture extracted with ether (2 x 200 ml). The ether extract was dried over anhydrous magnesium sulphate, passed through a pad of silica and the product eluted using ether.

The collected ether eluate was concentrated under reduced pressure to afford an oil which was purified using column chromatography (silica, hexane : ether (1:1)).

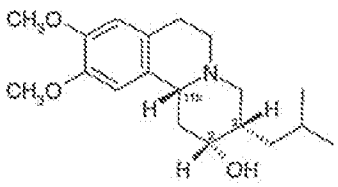
Evaporation of the collected column fractions of interest and removal of the solvent at reduced pressure gave a pink solid (6.53 g)

Preparative HPLC of the solid (Column: 2 x Lichrospher Si60, 5 μm, 250 x 21.20 mm; mobile phase hexane : isopropanol (97:3); UV 254 nm; flow: 10 ml min^{-1}) at 100 mg loading followed by concentration of the fractions of interest under vacuum gave a solid which was slurried with petroleum ether (30-40 °C) and collected by filtration to give the pure Mosher's ester derivatives

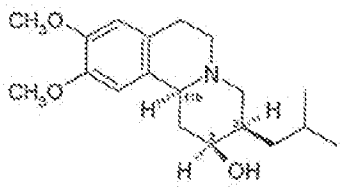
Peak 1 (2.37 g, 30%)

Peak 2 (2.42 g, 30%)

The fractions corresponding to the two peaks were subjected to hydrolysis to liberate the individual dihydrotetrabenazine isomers identified and characterised as Isomers C and D. Isomers C and D are each believed to have one of the following structures



2*R*,3*S*,11b*R*



2*S*,3*R*,11b*S*

2F. Hydrolysis of Peak 1 to give Isomer C

20% aqueous sodium hydroxide solution (53 ml) was added to a stirred solution of Mosher's ester peak 1 (2.37 g, 4.43 mmol) in methanol (158 ml) and the mixture stirred at reflux for 150 minutes. After cooling water (88 ml) was added to the reaction mixture and the resulting solution extracted with ether (576 ml). The organic extract was dried over anhydrous magnesium sulphate and after filtration the solvent removed at reduced pressure. Ethyl acetate (200 ml) was added to the residue and the solution washed with water (2 x 50 ml). The organic solution was dried over anhydrous magnesium sulphate and after filtration the solvent removed at reduced pressure.

This residue was treated with petroleum ether (30-40 °C) and the resulting suspended solid collected by filtration. The filtrate was concentrated at reduced pressure and the second batch of suspended solid was collected by filtration. Both collected solids were combined and dried under reduced pressure to give Isomer C (1.0 g, 70%).

Isomer C, which is believed to have either the 2*R*,3*S*,11b*R* or 2*S*,3*R*,11b*S* configuration (the absolute stereochemistry was not determined), was characterized by ^{1}H-NMR, ^{13}C-NMR, IR, mass spectrometry, chiral HPLC and ORD. The IR, NMR and MS data for Isomer C are set out in Table 2 and the Chiral HPLC and ORD data are set out in Table 4.

2G. Hydrolysis of Peak 2 to give Isomer D

20% aqueous sodium hydroxide solution (53 ml) was added to a stirred solution of Mosher's ester peak 2 (2.42 g, 4.52 mmol) in methanol (158 ml) and the mixture stirred at reflux for 150 minutes. After cooling water (88 ml) was added to the reaction mixture and the resulting solution extracted with ether (576 ml). The organic extract was dried over anhydrous magnesium sulphate and after filtration the solvent removed at reduced pressure. Ethyl acetate (200 ml) was added to the residue and the solution washed with water (2 x 50 ml). The organic solution was dried over anhydrous magnesium sulphate and after filtration the solvent removed at reduced pressure.

This residue was treated with petroleum ether (30-40 °C) and the resulting suspended orange solid collected by filtration. The solid was dissolved in ethyl acetate : hexane (15:85) and purified by column chromatography (silica, gradient ethyl acetate : hexane (15:85) to ethyl acetate). The fractions of interest were combined and the solvent removed at reduced pressure. The residue was slurried with petroleum ether (30-40 °C) and the resulting suspension collected by filtration. The collected solid was dried under reduced pressure to give Isomer D as a white solid (0.93 g, 64%).

Isomer D, which is believed to have either the 2*R*,3*S*,11b*R* or 2*S*,3*R*,11b*S* configuration (the absolute stereochemistry was not determined), was characterized

by ^{1}H-NMR, ^{13}C-NMR, IR, mass spectrometry, chiral HPLC and ORD. The IR, NMR and MS data for Isomer D are set out in Table 2 and the Chiral HPLC and ORD data are set out in Table 4.

In Tables 1 and 2, the infra red spectra were determined using the KBr disc method. The ^{1}H NMR spectra were carried out on solutions in deuterated chloroform using a Varian Gemini NMR spectrometer (200 MHz.). The ^{13}C NMR spectra were carried out on solutions in deuterated chloroform using a Varian Gemini NMR spectrometer (50MHz). The mass spectra were obtained using a Micromass Platform II (ES^{+} conditions) spectrometer. In Tables 3 and 4, the Optical Rotatory Dispersion figures were obtained using an Optical Activity PolAAr 2001 instrument in methanol solution at 24°C. The HPLC retention time measurements were carried out using an HP1050 HPLC chromatograph with UV detection.

Tables 1 and 2 – Spectroscopic Data

Table 1

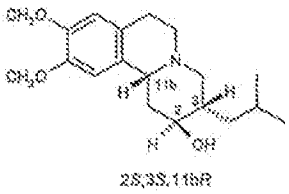
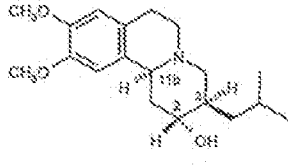
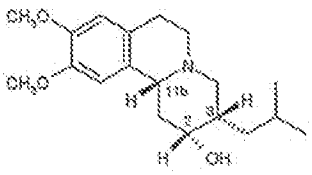
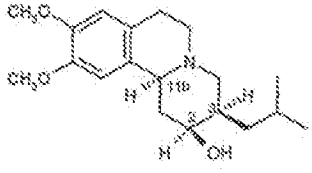
Dihydrotetrabenazine isomer	^{1}H-NMR spectrum ($CDCl_3$)	^{13}C-NMR spectrum ($CDCl_3$)	IR Spectrum (KBr solid)	Mass Spectrum (ES^{+})
Isomers A and B  2S,3S,11bR OR  2R,3R,11bS	6.67δ 1H (s); 6.57 δ 1H (s); 3.84 δ 6H (s); 3.55 δ 1H (br. d); 3.08 δ 1H (m); 2.79 δ 2H (m); 2.55 δ 3H (m); 2.17 δ 1H (m); 1.72 δ 6H (m); 1.02 δ 1H (m); 0.88 δ 6H (t)	147.7δ; 147.6δ; 130.5δ; 127.6δ; 112.1δ; 108.4δ; 70.5δ; 57.5 δ; 56.5 δ; 56.3 δ; 54.8 δ; 53.2 δ; 40.4 δ; 40.1 δ; 36.0 δ; 28.8 δ;	2950 cm^{-1}; 2928 cm^{-1}; 2868 cm^{-1}; 2834 cm^{-1}; 1610 cm^{-1}; 1511 cm^{-1}; 1464 cm^{-1} 1364 cm^{-1}; 1324 cm^{-1}; 1258 cm^{-1}; 1223 cm^{-1}; 1208 cm^{-1}; 1144 cm^{-1}; 1045 cm^{-1}; 1006 cm^{-1}; 870 cm^{-1};	MH^{+} 320

Table 1				
Dihydrotetrabenazine isomer	^{1}H-NMR spectrum ($CDCl_3$)	^{13}C-NMR spectrum ($CDCl_3$)	IR Spectrum (KBr solid)	Mass Spectrum (ES^+)
		26.2 δ; 23.78; 22.9 δ	785 cm^{-1}; 764 cm^{-1}	

Table 2				
Dihydrotetrabenazine isomer	^{1}H-NMR spectrum ($CDCl_3$)	^{13}C-NMR spectrum ($CDCl_3$)	IR Spectrum (KBr solid)	Mass Spectrum (ES^+)
Isomers C and D  2R,3S,11bR OR  2S,3R,11bS	6.68 δ 1H (s); 6.58 δ 1H (s); 3.92 δ 1H (m); 3.84 δ 6H (s); 3.15 δ 1H (m); 2.87 δ 3H (m); 2.43 δ 4H (m); 1.81 δ 1H (m); 1.64 δ 4H (m); 1.21 δ 1H (m); 0.94 δ 3H (d); 0.89 δ 3H (d)	147.8 δ; 147.7 δ; 130.4 δ; 127.2 δ; 112.0 δ; 108.3 δ; 72.4 δ; 61.2 δ; 58.3 δ; 56.5 δ; 56.3 δ; 52.7 δ; 38.6 δ; 36.7 δ; 34.4 δ; 29.6 δ; 26.5 δ; 24.4 δ; 22.5 δ	3370 cm^{-1}; 2950 cm^{-1}; 2929 cm^{-1}; 1611 cm^{-1}; 1512 cm^{-1}; 1463 cm^{-1} 1362 cm^{-1}; 1334 cm^{-1}; 1259 cm^{-1}; 1227 cm^{-1}; 1148 cm^{-1}; 1063 cm^{-1}; 1024 cm^{-1}; 855 cm^{-1}; 766 cm^{-1}	MH^+ 320

Tables 3 and 4 – Chromatography and ORD Data

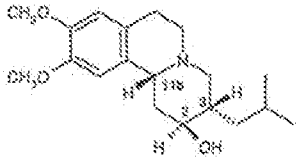
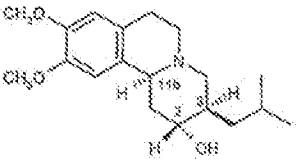
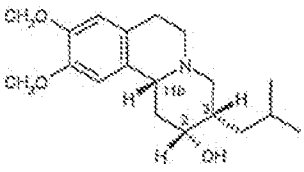
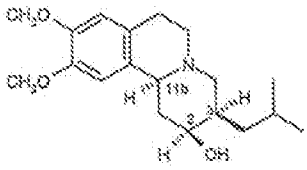
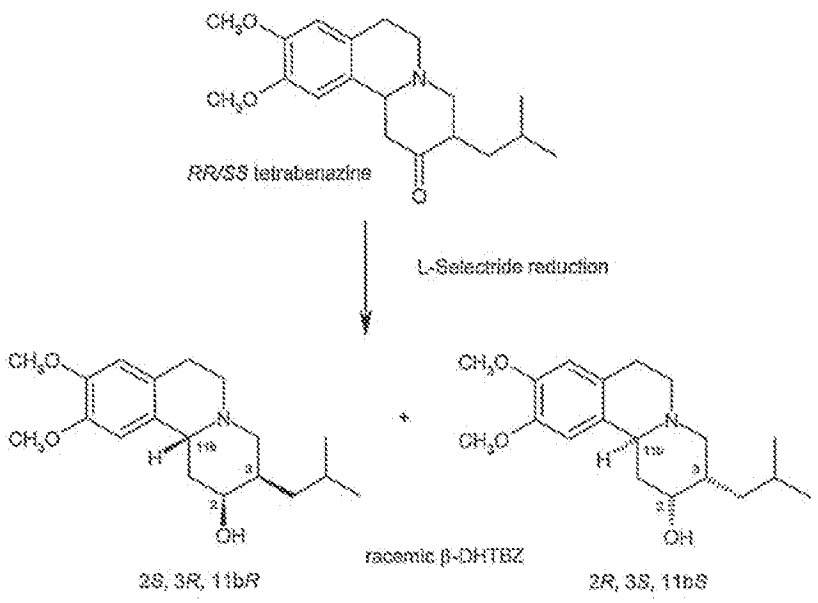
Table 3		
Dihydrotetrabenazine isomer	Chiral HPLC Methods and Retention Times	ORD (MeOH, 21°C)
Isomers A and B  2S,3S,11bR OR  2R,3R,11bS	Column: Chirex (S)-VAL, (R)-NEA, 250 x 4.6 mm Mobile phase: Hexane : 1,2-dichloroethane : ethanol (36:62:2) Flow: 1.0 ml min^{-1} UV: 254 nm Retention times: Isomer A 16.6 min Isomer B 15.3 min	Isomer A $[\alpha_D]$-114.6° Isomer B $[\alpha_D]$ +123°

Table 4		
Isomers C and D  2R,3S,11bR OR  2S,3R,11bS	Column: Chirex (S)-VAL, (R)-NEA, 250 x 4.6mm Mobile phase: Hexane : ethanol (92:8) Flow: 1.0 ml min^{-1} UV: 254 nm Retention times: Isomer C 20.3 min Isomer D 19.4 min	Isomer C $[\alpha_D]$ +150.9° Isomer D $[\alpha_D]$ -145.7°

EXAMPLE 3

Alternative Method of Preparation of Isomer B and Preparation of Mesylate Salt

3A. Reduction of *RR/SS* Tetrabenazine



1M L-Selectride® in tetrahydrofuran (52 ml, 52 mmol, 1.1 eq) was added slowly over 30 minutes to a cooled (ice bath), stirred solution of tetrabenazine racemate (15 g, 47 mmol) in tetrahydrofuran (56 ml). After the addition was complete, the mixture was allowed to warm to room temperature and stirred for a further six hours. TLC analysis (silica, ethyl acetate) showed only very minor amounts of starting material remained.

The mixture was poured on to a stirred mixture of crushed ice (112 g), water (56 ml) and glacial acetic acid (12.2 g). The resulting yellow solution was washed with ether (2 x 50 ml) and basified by the slow addition of solid sodium carbonate (ca. 13 g). Pet-ether (30-40 °C) (56 ml) was added to the mixture with stirring and the crude β-DHTBZ was collected as a white solid by filtration.

The crude solid was dissolved in dichloromethane (ca. 150 ml) and the resulting solution washed with water (40 ml), dried using anhydrous magnesium sulphate, filtered and concentrated at reduced pressure to ca. 40 ml. A thick suspension of white solid was formed. Pet-ether (30-40 °C) (56 ml) was added and the

suspension was stirred for fifteen minutes at laboratory temperature. The product was collected by filtration and washed on the filter until snow-white using pet-ether (30-40°C) (40 to 60 ml) before air-drying at room temperature to yield β-DHTBZ (10.1 g, 67%) as a white solid. TLC analysis (silica, ethyl acetate) showed only one component.

3B. Preparation and Fractional Crystallisation of the Camphorsulphonic acid Salt of Racemic β-DHTBZ

The product of Example 3A and 1 equivalent of (*S*)-(+)-Camphor-10-sulphonic acid were dissolved with heating in the minimum amount of methanol. The resulting solution was allowed to cool and then diluted slowly with ether until formation of the resulting solid precipitation was complete. The resulting white crystalline solid was collected by filtration and washed with ether before drying.

The camphorsulphonic acid salt of (10 g) was dissolved in a mixture of hot absolute ethanol (170 ml) and methanol (30 ml). The resulting solution was stirred and allowed to cool. After two hours the precipitate formed was collected by filtration as a white crystalline solid (2.9 g). A sample of the crystalline material was shaken in a separating funnel with excess saturated aqueous sodium carbonate and dichloromethane. The organic phase was separated, dried over anhydrous magnesium sulphate, filtered and concentrated at reduced pressure. The residue was triturated using pet-ether (30-40 °C) and the organic solution concentrated once more. Chiral HPLC analysis of the salt using a Chirex (S)-VAL and (R)-NEA 250 x 4.6 mm column, and a hexane : ethanol (98:2) eluent at a flow rate of 1 ml/minute showed showed that the isolated β-DHTBZ was enriched in one enantiomer (e.e. ca. 80%).

The enriched camphorsulphonic acid salt (14 g) was dissolved in hot absolute ethanol (140 ml) and propan-2-ol (420 ml) was added. The resulting solution was stirred and a precipitate began to form within one minute. The mixture was allowed to cool to room temperature and stirred for one hour. The precipitate formed was collected by filtration, washed with ether and dried to give a white crystalline solid (12 g).

The crystalline material was shaken in a separating funnel with excess saturated aqueous sodium carbonate and dichloromethane. The organic phase was separated, dried over anhydrous magnesium sulphate, filtered and concentrated at reduced pressure. The residue was triturated using pet-ether (30-40 °C) and the organic solution concentrated once more to yield (after drying in vacuo.) (+)-β-DHTBZ (6.6 g, ORD +107.8°). The isolated enantiomer has e.e. >97%.

3C. Preparation of Isomer B

A solution of phosphorus pentachloride (4.5 g, 21.6 mmol, 1.05 eq) in dichloromethane (55 ml) was added steadily over ten minutes to a stirred, cooled (ice-water bath) solution of the product of Example 3B (6.6 g, 20.6 mmol) in dichloromethane (90 ml). When the addition was complete, the resulting yellow solution was stirred for a further ten minutes before pouring on to a rapidly stirred mixture of sodium carbonate (15 g) in water (90 ml) and crushed ice (90 g). The mixture was stirred for a further 10 minutes and transferred to a separating funnel.

Once the phases had separated, the brown dichloromethane layer was removed, dried over anhydrous magnesium sulphate, filtered and concentrated at reduced pressure to give the crude alkene intermediate as brown oil (ca. 6.7 g). TLC analysis (silica, ethyl acetate) showed that no (+)-β-DHTBZ remained in the crude product.

The crude alkene was taken up (dry nitrogen atmosphere) in anhydrous tetrahydrofuran (40 ml) and a solution of borane in THF (1 M solution, 2.5 eq, 52 ml) was added with stirring over fifteen minutes. The reaction mixture was then stirred at room temperature for two hours. TLC analysis (silica, ethyl acetate) showed that no alkene intermediate remained in the reaction mixture.

A solution of sodium hydroxide (3.7 g) in water (10 ml) was added to the stirring reaction mixture, followed by an aqueous solution of hydrogen peroxide (50%, ca. 7 ml) and the two-phase mixture formed was stirred at reflux for one hour. TLC analysis of the organic phase at this time (silica, ethyl acetate) showed the

appearance of a product with Rf as expected for Isomer B. A characteristic non-polar component was also seen.

The reaction mixture was allowed to cool to room temperature and was poured into a separating funnel. The upper organic layer was removed and concentrated under reduced pressure to remove the majority of THF. The residue was taken up in ether (stabilised (BHT), 75 ml), washed with water (40 ml), dried over anhydrous magnesium sulphate, filtered and concentrated under reduced pressure to give a pale yellow oil (8.1 g).

The yellow oil was purified using column chromatography (silica, ethyl acetate : hexane (80:20), increasing to 100% ethyl acetate) and the desired column fractions collected, combined and concentrated at reduced pressure to give a pale oil which was treated with ether (stabilised, 18 ml) and concentrated at reduced pressure to give Isomer B as a pale yellow solid foam (2.2 g).

Chiral HPLC using the conditions set out in Example 3B confirmed that Isomer B had been produced in an enantiomeric excess (e.e.) of greater than 97%.

The optical rotation was measured using a Bellingham Stanley ADP220 polarimeter and gave an $[\alpha_D]$ of +123.5°.

3D. Preparation of the Mesylate salt of Isomer B

The methanesulphonate salt of Isomer B was prepared by dissolving a mixture of 1 equivalent of Isomer B from Example 3C and 1 equivalent of methane sulphonic acid in the minimum amount of ethanol and then adding diethyl ether. The resulting white precipitate that formed was collected by filtration and dried *in vacuo* to give the mesylate salt in a yield of ca. 85% and a purity (by HPLC) of ca. 96%.

EXAMPLE 4

X-Ray Crystallographic Studies on Isomer B

The (*S*)-(+)-Camphor-10-sulphonic acid salt of Isomer B was prepared and a single crystal was subjected to X-ray crystallographic studies under the following conditions:

Diffractometer: Nonius KappaCCD area detector (f/i scans and OJ scans to fill asymmetric unit).

Cell determination: DirAx (Duisenberg, A.J.M.(1992). *J. Appl. Cryst.* 25, 92-96.)

Data collection: Collect (Collect: Data collection software, R. Hooft, Nonius B. V, 1998)

Data reduction and cell refinement: Demo (Z. Otwinowski & W. Minor, *Methods in Enzymology* (1997) Vol. 276: *Macromolecular Crystallography*, part A, pp. 307-326; C. W. Carter, Jr & R. M. Sweet, Eds., Academic Press).

Absorption correction: Sheldrick, G. M. SADABS - Bruker Nonius area detector scaling and absorption correction - V2.\ 0

Structure solution: SHELXS97 (G. M. Sheldrick, *Acta Cryst.* (1990) A46 467-473). Structure refinement: SHELXL97 (G. M. Sheldrick (1997), University of Göttingen, Germany)

Graphics: Cameron - A Molecular Graphics Package (D. M. Watkin, L. Pearce and C. K. Prout, Chemical Crystallography Laboratory, University of Oxford,1993)

Special details: All hydrogen atoms were placed in idealised positions and refined using a riding model, except those of the NH and OH which were located in the difference map and refined using restraints. Chirality: NI=R, CI2=S, CI3=S, CI5=R, C21=S, C24=R

The results of the studies are set out below in Tables A, B, C, D and E.

In the Tables, the label RUS0350 refers to Isomer B.

TABLE A

Identification code	**2005bdy0585 (RUS0350)**
Empirical formula	$C_{28}H_{45}NO_7S$
Formula weight	551.72
Temperature	120(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	a = 7.1732(9) Å
	b = 12.941(2) Å
	c = 31.025(4) Å
Volume	2880.1(7) Å^3
Z	4
Density (calculated)	1.272 Mg / m^3
Absorption coefficient	0.158 mm^{-1}
$F(000)$	1192
Crystal	Colourless Slab
Crystal size	0.2 × 0.2 × 0.04 mm^3
θ range for data collection	3.06 – 27.37°
Index ranges	$-8 \le h \le 9$, $-16 \le k \le 16$, $-36 \le l \le 39$
Reflections collected	36802
Independent reflections	6326 [R_{int} = 0.0863]
Completeness to θ = 27.37°	97.1 %
Absorption correction	Semi–empirical from equivalents
Max. and min. transmission	0.9937 and 0.9690
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	6326 / 1 / 357
Goodness-of-fit on F^2	1.042
Final R indices [$F^2 > 2\sigma(F^2)$]	$R1$ = 0.0498, $wR2$ = 0.0967
R indices (all data)	$R1$ = 0.0901, $wR2$ = 0.1108
Absolute structure parameter	0.04(8)
Extinction coefficient	0.0059(7)
Largest diff. peak and hole	0.236 and –0.336 e Å^{-3}

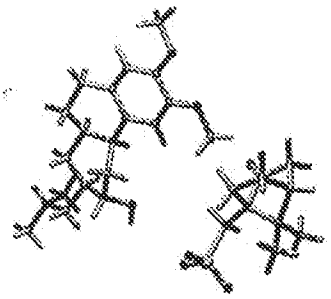


TABLE B. Atomic coordinates [x 10^4], equivalent isotropic displacement parameters [Å^2 x 10^3] and site occupancy factors. U_{eq} is defined as one third of the trace of the orthogonalized U^{ij} tensor.

Atom	x	y	z	Ueq	S.o.f.
Ni	4839(3)	11119(2)	2180(1)	24(1)	1
O1	2515(3)	13171(1)	349(1)	31(1)	1
O2	5581(3)	14030(1)	598(1)	32(1)	1
O3	9220(3)	12834(2)	2385(1)	36(1)	1
C1	870(4)	12674(2)	190(1)	36(1)	1
C2	3176(3)	12838(2)	739(1)	25(1)	1

C3	2346(4)	12109(2)	997(1)	25(1)	1
C4	3124(3)	11821(2)	1395(1)	24(1)	1
C5	4773(3)	12276(2)	1527(1)	23(1)	1
C6	5629(4)	13024(2)	1262(1)	24(1)	1
C7	4861(4)	13308(2)	875(1)	25(1)	1
C8	7189(4)	14582(2)	747(1)	38(1)	1
C9	2182(3)	11023(2)	1673(1)	28(1)	1
C10	2759(3)	11118(2)	2137(1)	26(1)	1
C11	5366(3)	11096(2)	2656(1)	25(1)	1
C12	7292(4)	11536(2)	2747(1)	25(1)	1
C13	7468(4)	12663(2)	2590(1)	25(1)	1
C14	5988(4)	12911(2)	2252(1)	25(1)	1
C15	5773(4)	12010(2)	1943(1)	24(1)	1
C16	7734(4)	11477(2)	3232(1)	28(1)	1
C17	7752(4)	10418(2)	3449(1)	34(1)	1
C18	9198(6)	9696(3)	3249(1)	65(1)	1
C19	8114(4)	10562(2)	3930(1)	41(1)	1
C20	7509(4)	8131(2)	1250(1)	31(1)	1
S1	7409(1)	8792(1)	1754(1)	27(1)	1
04	7758(2)	7965(1)	2064(1)	30(1)	1
05	8831(3)	9582(2)	1760(1)	49(1)	1
06	5524(2)	9221(1)	1798(1)	32(1)	1
07	7406(3)	6932(1)	498(1)	48(1)	1
C21	6858(3)	8622(2)	830(1)	25(1)	1
C22	7154(4)	7851(2)	459(1)	30(1)	1
C23	7073(4)	8450(2)	40(1)	32(1)	1
C24	6648(3)	9544(2)	203(1)	28(1)	1
C25	4742(3)	8877(2)	787(1)	29(1)	1
C26	4742(3)	8877(2)	787(1)	29(1)	1
C27	7773(4)	9610(2)	630(1)	25(1)	1
C28	7431(4)	10628(2)	868(1)	29(1)	1
C29	9895(4)	9489(2)	569(1)	36(1)	1

TABLE C. Bond lengths [A] and angles [°].

NI-C10	1.498(3)	C14-C15	1.518(3)
NI-C15	1.522(3)	C16-C17	1.526(3)
NI-C11	1.524(3)	C17-C18	1.527(4)
01-C2	1.368(3)	C17-C19	1.527(4)
O1-C1	1.432(3)	C20-C21	1.525(3)
02-C7	1.369(3)	C20-S1	1.784(2)
02-C8	1.433(3)	S1-05	1.4442(19)
03-C13	1.425(3)	S1-04	1.4607(17)
C2-C3	1.372(3)	S1-06	1.4676(18)
C2-C7	1.417(3)	07-C22	1.208(3)
C3-C4	1.407(3)	C21-C22	1.537(4)
C4-C5	1.384(3)	C21-C26	1.559(3)
C4-C9	1.506(3)	C21-C27	1.565(3)

	C5-C6	1.411(3)	C22-C23	1.517(4)
	C5-C15	1.516(3)	C23-C24	1.535(4)
	C6-C7	1.372(3)	C24-C25	1.548(4)
	C9-C10	1.504(3)	C24-C27	1.554(4)
	C11-C12	1.521(3)	C25-C26	1.557(4)
	C12-C16	1.540(3)	C27-C28	1.529(3)
	C12-C13	1.544(3)	C27-C29	1.542(4)
	C13-C14	1.524(3)		
	C10-N1-C15	113.33(19)	C12-C11-N1	113.43(19)
	C10-N1-C11	109.46(18)	C11-C12-C16	110.5(2)
	C15-N1-C11	111.96(19)	C11-C12-C13	111.7(2)
	C2-01-C1	116.6(2)	C16-C12-C13	109.84(19)
	C7-02-C8	116.27(19)	03-C13-C14	106.0(2)
	01-C2-C3	125.5(2)	03-C13-C12	111.1(2)
	01-C2-C7	115.0(2)	C14-C13-C12	111.0(2)
	C3-C2-C7	119.5(2)	C15-C14-C13	110.1(2)
	C2-C3-C4	121.5(2)	C5-C15-C14	114.3(2)
	C5-C4-C3	119.2(2)	C5-C15-N1	112.0(2)
	C5-C4-C9	120.3(2)	C14-C15-N1	108.7(2)
	C3-C4-C9	120.5(2)	C17-C16-C12	118.4(2)
	C4-C5-C6	119.4(2)	C16-C17-C18	112.2(2)
	C4-C5-C15	124.1(2)	C16-C17-C19	108.7(2)
	C6-C5-C15	116.6(2)	C18-C17-C19	110.8(3)
	C7-C6-C5	121.3(2)	C21-C20-S1	122.51(18)
	02-C7-C6	125.4(2)	05-SI-04	112.93(11)
	02-C7-C2	115.4(2)	05-SI-06	112.47(12)
	C6-C7-C2	119.2(2)	04-SI-06	111.93(11)
	C10-C9-C4	111.7(2)	05-SI-C20	108.81(13)
	N1-C10-C9	111.0(2)	04-SI-C20	102.60(11)
	06-SI-C20	107 .44(12)	C23-C24-C25	106.4(2)
	C20-C21-C22	109.0(2)	C23-C24-C27	103.3(2)
	C20-C21-C26	117.3(2)	C25-C24-C27	102.3(2)
	C22-C21-C26	102.1(2)	C24-C25-C26	102.9(2)
	C20-C21-C27	123.4(2)	C25-C26-C21	104.2(2)
	C22-C21-C27	100.21(19)	C28-C27-C29	107.8(2)
	C26-C21-C27	101.7(2)	C28-C27-C24	112.0(2)
	07-C22-C23	126.4(2)	C29-C27-C24	113.7(2)
	07-C22-C21	125.9(2)	C28-C27-C21	116.5(2)
	C23-C22-C21	107.7(2)	C29-C27-C21	112.3(2)
	C22-C23-C24	101.3(2)	C24-C27-C21	94.27(19)

TABLE D. Anisotropic displacement parameters [$A^2 \times 10^3$]. The anisotropic displacement factor exponent takes the form:- $2\pi^2[h^2a^{*2}U^{11} + ... + 2\,h\,k\,a^*\,b^*\,U^{12}]$.

Atom	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}

N1	26(1)	24(1)	23(1)	2(1)	-1(1)	-3(1)
O1	37(1)	30(1)	24(1)	3(1)	-7(1)	-4(1)
O2	41(1)	31(1)	25(1)	5(1)	-2(1)	-10(1)
O3	26(1)	49(1)	32(1)	7(1)	-3(1)	-9(1)
Cl	41(2)	36(2)	32(2)	3(1)	-9(1)	-8(2)
C2	30(2)	24(2)	22(1)	1(1)	-1(1)	2(1)
C3	25(1)	26(1)	24(1)	-3(1)	-2(1)	2(1)
C4	26(2)	22(1)	23(1)	-1(1)	2(1)	-1(1)
C5	24(1)	22(1)	23(1)	-2(1)	1(1)	0(1)
C6	26(1)	22(1)	24(1)	-3(1)	2(1)	-5(1)
C7	30(2)	22(1)	22(1)	2(1)	4(1)	-4(1)
C8	45(2)	34(2)	36(2)	5(1)	-2(1)	-20(2)
C9	23(1)	32(1)	29(2)	3(1)	-1(1)	-4(1)
ClO	26(1)	29(1)	25(1)	2(1)	0(1)	-5(1)
C11	31(1)	25(1)	20(1)	2(1)	0(1)	-2(1)
C12	26(1)	26(1)	23(1)	-1(1)	1(1)	-1(1)
Cl3	26(1)	28(1)	23(1)	-1(1)	-1(1)	-2(1)
Cl4	30(2)	22(2)	24(1)	-1(1)	1(1)	-1(1)
Cl5	22(1)	22(1)	28(1)	2(1)	0(1)	-4(1)
C16	31(1)	28(1)	24(1)	-1(1)	-3(1)	3(1)
Cl7	46(2)	31(2)	25(1)	1(1)	-7(1)	0(2)
Cl8	106(3)	46(2)	41(2)	6(2)	-1(2)	31(2)
C19	51(2)	41(2)	31(2)	9(2)	-7(1)	-4(2)
C20	30(2)	34(2)	29(1)	2(1)	3(1)	9(2)
S1	27(1)	30(1)	24(1)	4(1)	-2(1)	-5(1)
O4	31(1)	36(1)	23(1)	9(1)	-1(1)	0(1)
O5	53(1)	58(1)	37(1)	13(1)	-11(1)	-35(1)
O6	34(1)	35(1)	28(1)	-3(1)	-2(1)	10(1)
O7	81(2)	25(1)	40(1)	-1(1)	12(1)	6(1)
C21	26(1)	25(2)	24(1)	-1(1)	3(1)	2(1)
C22	35(2)	25(2)	31(2)	0(1)	1(1)	-1(1)
C23	40(2)	30(2)	25(1)	-2(1)	1(1)	-2(1)
C24	28(1)	29(2)	26(2)	2(1)	2(1)	2(1)
C25	30(2)	34(2)	29(2)	-1(1)	-2(1)	0(1)
C26	26(1)	34(2)	28(2)	0(1)	1(1)	-5(1)
C27	23(1)	26(1)	26(1)	0(1)	2(1)	0(1)
C28	31(1)	26(1)	30(1)	0(1)	-2(1)	-6(1)
C29	29(2)	41(2)	40(2)	0(2)	2(1)	-3(1)

TABLE E. Hydrogen coordinates [x 10^4] and isotropic displacement parameters [A^2 x 10^3].

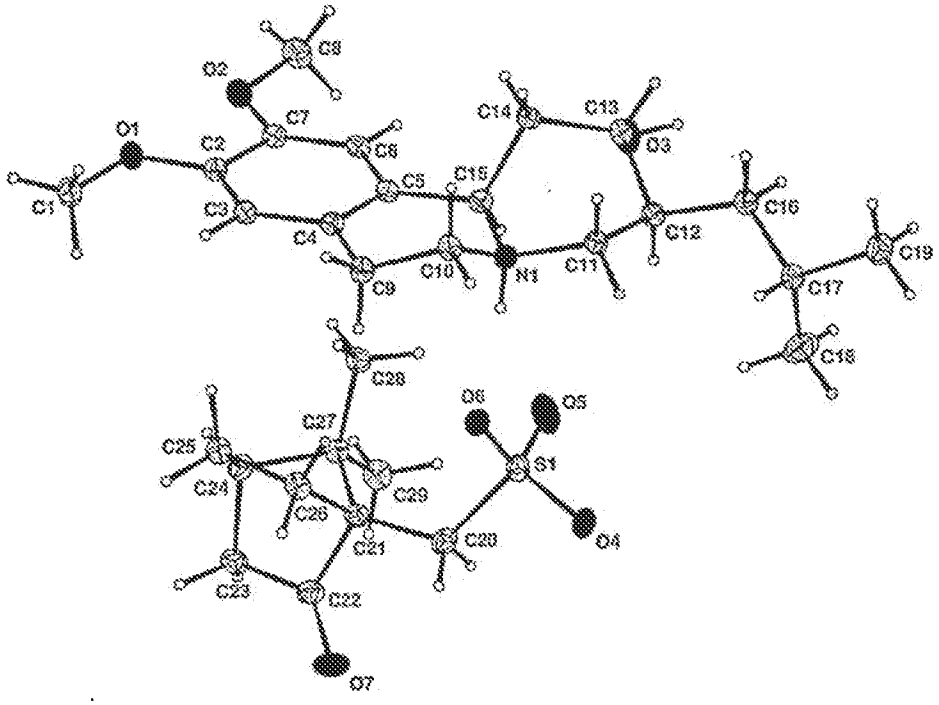
Atom	x	y	z	U_{eq}	S.o.f
H98	5190(40)	10528(15)	2062(10)	70(8)	1
H99	10030(50)	12950(30)	2575(12)	70(8)	1
H1A	1107	11933	156	54	1
H1B	529	12973	-69	54	1

	H1C	-154	12777	395	54	1
	H3	1220	11793	904	30	1
	H6	6760	13337	1353	29	1
	H8A	6872	14966	1009	58	1
5	H8B	7600	15065	523	58	1
	H8C	8193	14091	810	58	1
	H9A	814	11106	1651	33	1
	H9B	2505	10324	1567	33	1
	H10A	2250	11767	2259	32	1
10	H10B	2235	10534	2304	32	1
	H11A	4431	11494	2822	30	1
	H11B	5322	10372	2759	30	1
	H12	8230	11108	2589	30	1
	H13	7334	13145	2840	30	1
15	H14A	4783	13050	2397	30	1
	H14B	6354	13538	2090	30	1
	H15	7056	11776	1864	29	1
	H16A	8973	11796	3276	33	1
	H16B	6813	11911	3386	33	1
20	I H17	6493	10098	3412	41	1
	H18A	8906	9588	2944	97	1
	H18B	9176	9031	3400	97	1
	H18C	10440	10005	3276	97	1
	H19A	9329	10894	3971	62	1
25	H19B	8110	9887	4073	62	1
	H19C	7135	10999	4054	62	1
	H20A	8824	7924	1207	37	1
	H20B	6787	7484	1286	37	1
	H23A	6070	8190	-151	38	1
30	H23B	8277	8423	-116	38	1
	H24	6928	10107	-8	33	1
	H25A	3773	9195	153	37	1
	H25B	4152	10235	426	37	1
	H26A	3994	8237	764	35	1
35	H26B	4300	9279	1039	35	1
	H28A	8160	10638	1135	44	1
	I H28B	6103	10692	936	44	1
	H28C	7811	11207	684	44	1
	H29A	10358	10042	381	54	1
40	H29B	10159	8817	436	54	1
	H29C	10517	9531	849	54	1

Table 6. Hydrogen bonds [Å and °].

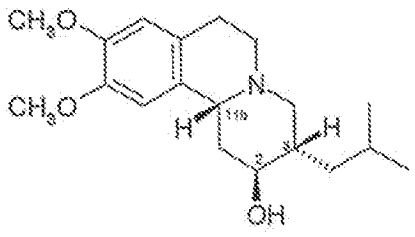
D–H···*A*	*d*(*D*–H)	*d*(H···*A*)	*d*(*D*···*A*)	∠(*DHA*)
N1–H98···O6	0.885(10)	1.895(12)	2.773(3)	171(3)
N1–H98···S1	0.885(10)	2.914(14)	3.771(2)	163(3)
O3–H99···O4^{i}	0.84(4)	1.94(4)	2.766(3)	165(3)
O3–H99···S1^{i}	0.84(4)	2.98(4)	3.811(2)	169(3)

Symmetry transformations used to generate equivalent atoms:
(i) –x+2,y+1/2,–z+1/2



Thermal ellipsoids drawn at the 30% probability level

On the basis of the data set out above, Isomer B is believed to have the 2S, 3S, 11bR configuration, which corresponds to Formula (Ia):



(Ia) – Isomer B

EXAMPLE 5

Analysis of the effect of Isomer B in a transgenic mice model of Huntington's disease

B6CBA-Tg(HDexon1)62Gpb/1J transgenic (R6/2) mice are transgenic for the 5-end of the human HD gene carrying (CAG) 115 - (CAG)150 repeat expansions. R6/2 transgenic mice exhibit a progressive neurological phenotype that mimics many of the features of Huntington's disease, including choreiform-like movements, involuntary stereotypic movements, tremor and epileptic seizures. They urinate frequently and exhibit loss of body weight through the course of the disease. These symptoms appear between 6 and 8 weeks of age.

A study was carried out to assess the effect of Isomer B in this transgenic mouse model of Huntington's disease by assessing animals in a battery of behavioural tests.

Methods

Female B6CBa-Tg(HDexon1)62Gpb/1J transgenic mice (Jackson Laboratory, USA) were housed 5 per cage in an enriched environment under a light-dark cycle of 12 h – 12 h (light on at 7.00 am, off at 7.00 pm) at a room temperature of 21 ± 2 °C, with 50±15% humidity. The mice had access to commercial mouse chow (mouse/rats breeding, ref. 9341 Provimi Kliba, Switzerland) and tap water.

Isomer B in corn oil was administered repeatedly (5 mg/kg i.p) once per day for 4 days to 10 weeks old mice.

On the testing days, animals were subjected to the following tests, using the protocol described in the protocol section:

1. Simplified Irwin test

Two hours before the observations, animals were placed in individual cages. Measurements were first carried out in the individual cages. The animal's convulsions, tremors-twitches, stereotypies and vocalizations were recorded. The animal was then placed in a 58.5 x 68.5 cm open field with a 6 cm rim and observed for approximately 3 minutes. Gait characteristics were ranked. The presence of convulsions or tremors-twitches and stereotypies was again noted. At the end of the 3 minutes, the number of faecal boluses and pools of urine was recorded. The mice were then returned to their individual cages after testing.

2. Locomotor activity

The mice were placed in a transparent plastic box of floor dimensions 30 x 30 cm in a room with low light intensity (maximum 20 lux). Locomotor activity was determined during a 10 minute period using a video image analyzer (Videotrack, View Point, Lyon, France). The number, distance and average speed of ambulatory movements were measured. The mice were returned to their home cages after testing.

3. Rotarod

Two consecutive days before the first administration, animals were trained to use the rotarod: they were placed on an accelerating rotarod (Ugo Basile, Italy) for a maximum time of 450 seconds. They went through 2 training sessions starting with 4 rpm for 300 seconds, then staying at 40 rpm for 150 seconds. The two training sessions were given at 1 hour intervals. On the days of testing, each animal was subjected to one trial under the conditions described above. Each trial was terminated when the mouse fell or when it had stayed on the rotarod for 450 seconds. The mice were returned to their home cages after testing.

The results of the tests are shown in Tables 5 to 9.

Experimental protocol

Size of experimental groups: 10

Group 1: hemizygote Transgenic B6CBA-Tg(HDexon 1)62Gpb/1J mice treated with vehicle i.p. once a day for 4 days (from day 0 to day 3)

Group 2: hemizygote Transgenic B6CBA-Tg(HDexon 1)62Gpb/1J mice treated with 5 mg/kg i.p. of test item once a day for 4 days (from day 0 to day 3)

Testing protocol

At the age of 10 weeks, before administration (Day 0) and each day for 3 days following administration, animals were subject to the tests described below, as follows:

Day -2

- Rotarod training, 2 sessions at 1 hour interval

Day -1

- Rotarod training, 2 sessions at 1 hour interval

Day 0

- Simplified Irwin test
- Locomotor activity test, immediately after the simplified Irwin test
- Rotarod test, immediately after the locomotor activity test
- Test item administration, 1 hour after the rotarod test
- Simplified Irwin test, 40 minutes after administration
- Locomotor activity, immediately after the simplified Irwin test

Day 1

- Test item administration
- Simplified Irwin test, 40 minutes after administration
- Rotarod test, immediately after the simplified Irwin test

Day 2

- Test item adminstation
- Simplified Irwin test, 40 min after administration
- Rotarod test, immediately after simplified #Irwin test

Day 3

- Test item administration
- Simplified Irwin test, 40 min after administration
- Locamotor activity test, immediately after the simplified Irwin test

Statistical analysis

Simplified Irwin scores were analysed using non-parametric Mann-Whitney's U test. Locomotor activity data were analysed using Dunnett's t-test. Rotarod scores were analysed using the non-parametric Mann-Whitney's U-test. Statistical analyses were performed using the software Statview SE$^{+graphics}$ software, Brain Power.

Table 5- Effects of Isomer B in a transgenic mice model of Huntington's disease
Phase I : Efficacy in acute study, administration from Day 0 to Day 3 - Simplified Irwin test

Corn oil, 10 ml/kg i.p.	Day 0 before administration	Day 0 after administration	Day 1	Day 2	Day 3	Day 17	Day 21	Day 24	Day 27	Day 31
HOME CAGE										
Convulsions	—	—	—	0.78 ± 0.40	0.67 ± 0.44	X	X	X	X	X
Tremors-Twitches	1.33 ± 0.17	1.00 ± 0.27	0.78 ± 0.22	0.88 ± 0.13	1.44 ± 0.29	X	X	X	X	X
Stereotypies	—	—	—	0.11 ± 0.11	—	X	X	X	X	X
Grooming (sec)	21.00 ± 8.49	4.00 ± 4.00	1.33 ± 1.33	14.22 ± 8.18	11.67 ± 7.72	X	X	X	X	X
Vocalization	—	—	—	—	—	X	X	X	X	X
OPEN FIELD								n=2/8	n=2/8	n=3/7
Convulsions	—	—	—	—	—	—	—	—	-	—
Tremors-Twitches	2.11 ± 0.26	1.89 ± 0.11	2.22 ± 0.15	1.89 ± 0.20	1.67 ± 0.24	2.78 ± 0.15	2.69 ± 0.29	3.38 ± 0.25	2.00 ± 0.25	2.71 ± 0.25
Stereotypies	—	—	—	—	—	—	—	—	—	—

Grooming (sec)	18.89 ± 10.50	40.89 ± 15.82	26.00 ± 13.43	45.67 ± 16.53	44.33 ± 9.07	24.67 ± 8.18	20.63 ± 8.90	29.50 ± 8.67	30.63 ± 12.5	21.29 ± 9.53
Gait	1.22 ± 0.46	0.89 ± 0.35	0.56 ± 0.29	1.33 ± 0.47	2.33 ± 0.24	2.89 ± 0.11	2.75 ± 0.15	2.88 ± 0.28	2.25 ± 0.50	3.14 ± 0.30
Vocalisation	—	—	—	—	—	0.22 ± 0.22	0.50 ± 0.31	0.25 ± 0.24	0.50 ± 0.31	1.14 ± 0.36
Defecation (N° of boluses)	1.67 ± 0.50	1.33 ± 0.29	1.00 ± 0.33	1.67 ± 0.29	2.22 ± 0.40	1.11 ± 0.56	1.75 ± 0.43	1.35 ± 0.39	1.50 ± 0.31	1.57 ± 0.42
Urination (N° of pools)	0.44 ± 0.24	0.22 ± 0.15	0.33 ± 0.24	0.22 ± 0.15	0.11 ± 0.11	0.22 ± 0.15	0.50 ± 0.18	0.13 ± 0.12	0.00 ± 0.00	0.00 ± 0.00

Isomer B 5 mg/kg i.p.	Day 0 before administration	Day 0 after administration	Day 1	Day 2	Day 3	Day 17	Day 21	Day 24	Day 27	Day 31
HOME CAGE										
Convulsions	0.22 ± 0.22	0.13 ± 0.13	—	—	0.11 ± 0.11	X	X	X	X	X
Tremors-Twitches	1.44 ± 0.24	1.13 ± 0.30	1.00 ± 0.24	0.78 ± 0.15	1.00 ± 0.29	X	X	X	X	X
Stereotypies	—	—	—	—	—	X	X	X	X	X
Grooming (sec)	10.00 ± 7.07	7.38 ± 6.54	—	7.78 ± 6.13	4.22 ± 4.22	X	X	X	X	X

Vocalization	----	----	----	----	----	X	X	X	X	X
OPEN FIELD										
Convulsions	----	0.67 ± 0.67	0.11 ± 0.11	----	----	0.67 ± 0.67	----	----		----
Tremors-Twitches	2.11 ± 0.11	1.89 ± 0.26	2.33 ± 0.17	2.00 ± 0.24	1.78 ± 0.36	1.44 ± 0.24*	1.67 ± 0.22*	2.44 ± 0.18*	2.11 ± 0.39	2.67 ± 0.24
Stereotypies	----	0.11 ± 0.11	----	----	----	----	----	----	----	----
Grooming (sec)	19.44 ± 6.92	3.11 ± 2.45	7.78 ± 4.34	20.56 ± 6.94	12.67 ± 6.71**	20.33 ± 7.49	17.67 ± 6.33	18.22 ± 8.25	25.33 ± 8.14	19.11 ± 8.71
Gait	1.11 ± 0.39	0.33 ± 0.24	1.00 ± 0.33	0.67 ± 0.37	2.33 ± 0.33	1.56 ± 0.34*	1.67 ± 0.37*	2.17 ± 0.12*	2.56 ± 0.24	2.33 ± 0.29
Vocalisation	0.44 ± 0.29	0.22 ± 0.22	0.67 ± 0.33*	0.89 ± 0.35	0.89 ± 0.35*	0.22 ± 0.22	0.44 ± 0.29	0.44 ± 0.29	0.00 ± 0.00	0.22 ± 0.22
Defecation (N° of boluses)	2.56 ± 0.50	1.44 ± 0.50	0.89 ± 0.26	1.22 ± 0.43	1.33 ± 0.33	2.67 ± 0.76	2.44 ± 0.53	1.33 ± 0.55	2.33 ± 0.58	1.44 ± 0.38
Urination (N° of pools)	0.67 ± 0.44	0.44 ± 0.34	0.11 ± 0.11	0.33 ± 0.24	0.11 ± 0.11	0.22 ± 0.15	0.11 ± 0.11	0.33 ± 0.17	0.44 ± 0.18*	0.11 ± 0.11

---- : Sign never observed (equivalent to 0.00 ± 0.00) a : p = 0.06 versus control group, Mann-Whitney U-test

X : Observation not performed * ; ** : Significantly different from control group ($p<0.05$; $p<0.01$), Mann-Whitney U-test

Table 6

Effects of Isomer B in a transgenic mice model of Huntington's disease

Phase I : Efficacy in acute study - Locomotor activity test

Average recorded results

DAY 0, before administration

	Inactivity	Large movements		
Treatment	*Duration*	*Occurence*	*Duration*	*Speed*
	(sec)	*(number)*	*(sec)*	*(cm/sec)*
Corn oil, 10 mL/kg i.p.	140 ± 17	292 ± 34	126 ± 17	14.2 ± 0.8
RUS-350, 5 mg/kg i.p.	149 ± 27	309 ± 37	128 ± 16	13.2 ± 0.5

DAY 0, 40 mn after administration

	Inactivity	Large movements		
Treatment	*Duration*	*Occurence*	*Duration*	*Speed*
	(sec)	*(number)*	*(sec)*	*(cm/sec)*
Corn oil, 10 mL/kg i.p.	199 ± 16	205 ± 14	84 ± 8	14.0 ± 0.7
RUS-350, 5 mg/kg i.p.	184 ± 31	280 ± 41	99 ± 13	13.0 ± 0.5

DAY 2, 40 mn after administration

	Inactivity	Large movements		
Treatment	*Duration*	*Occurence*	*Duration*	*Speed*
	(sec)	*(number)*	*(sec)*	*(cm/sec)*
Corn oil, 10 mL/kg i.p.	171 ± 15	245 ± 22	104 ± 9	14.1 ± 0.7
RUS-350, 5 mg/kg i.p.	134 ± 18	322 ± 40	123 ± 14	13.9 ± 0.7

Table 7

Effects of Isomer B in a transgenic mice model of Huntington's desease

Phase I : Efficacy in acute study - Locomotor activity test

Average results relative to pre-administration

DAY 0, before administration

	Inactivity	Large movements

Treatment	*Duration*	*Occurence*	*Duration*	*Speed*
	(sec)	*(number)*	*(sec)*	*(cm/sec)*
Corn oil, 10 mL/kg i.p.	0 ± 0	0 ± 0	0 ± 0	0.0 ± 0.0
RUS-350, 5 mg/kg i.p.	0 ± 0	0 ± 0	0 ± 0	0.0 ± 0.0

DAY 0, 40 mn after administration

	Inactivity	**Large movements**		
Treatment	*Duration*	*Occurence*	*Duration*	*Speed*
	(sec)	*(number)*	*(sec)*	*(cm/sec)*
Corn oil, 10 mL/kg i.p.	60 ± 20	-87 ± 24	-42 ± 10	-0.2 ± 0.4
RUS-350, 5 mg/kg i.p.	36 ± 30	-29 ± 41	-29 ± 15	-0.1 ± 0.5

DAY 2, 40 mn after administration

	Inactivity	**Large movements**		
Treatment	*Duration*	*Occurence*	*Duration*	*Speed*
	(sec)	*(number)*	*(sec)*	*(cm/sec)*
Corn oil, 10 mL/kg i.p.	31 ± 10	-47 ± 23	-21 ± 11	-0.1 ± 0.7
RUS-350, 5 mg/kg i.p.	-15 ± 27	13 ± 42	-6 ± 16	0.7 ± 0.7

Table 8

Effects of Isomer B in a transgenic mice model of Huntington's desease

Phase I : Efficacy in acute study - Locomotor activity test

Average recorded results

DAY 24

	Inactivity	**Large movements**		
Treatment	*Duration*	*Occurence*	*Duration*	*Speed*
	(sec)	*(number)*	*(sec)*	*(cm/sec)*
Corn oil, 10 mL/kg i.p.	36 ± 20	741 ± 152	136 ± 22	11.8 ± 0.6
RUS-350, 5 mg/kg i.p.	67 ± 42	818 ± 168	148 ± 34	11.2 ± 0.9

Table 9

Effects of Isomer B in a transgenic mice model of Huntington's desease

Phase I : Efficacy in acute study, administration from Day 0 to Day 3 - Rotarod test

Measured latencies to fall from Rotarod (sec) after daily administration of Isomer B		
	Corn oil, 10 mL/kg i.p.	Isomer B, 5 mg/kg i.p.
Day 0, prior to 1st administration	76.9 ± 16.6	68.4 ± 17.7
Day 1	76.2 ± 18.3	91.4 ± 18.1
Day 3	52.9 ± 15.7	59.4 ± 11.2
Day 9	46.0 ± 13.1	29.0 ± 12.1
Day 17	26.0 ± 9.2	30.0 ± 11.0
Day 21	18.5 ± 8.6	22.1 ± 7.3
Day 24	20.9 ± 11.1	14.8 ± 4.3
Day 27	20.1 ± 11.9	15.8 ± 5.2
Day 31	16.1 ± 9.8	7.8 ± 2.4

Latencies relative to Day 0 (prior to 1st administration)		
	Corn oil, 10 mL/kg i.p.	Isomer B, 5 mg/kg i.p.
Day 1 - Day 0	-0.7 ± 13.0	23.0 ± 9.8
Day 3 - Day 0	-24.0 ± 10.9	-9.0 ± 11.9
Day 9 - Day 0	-32.1 ± 9.7	-39.7 ± 9.9
Day 17 - Day 0	-51.3 ± 15.2	-38.2 ± 13.1
Day 21 - Day 0	-64.3 ± 13.3	-46.3 ± 15.5
Day 24 - Day 0	-61.9 ± 12.0	-53.7 ± 16.8
Day 27 - Day 0	-62.6 ± 13.1	-52.7 ± 16.2
Day 31 - Day 0	-72.3 ± 16.4	-60.7 ± 16.2

The results demonstrate that although both control mice and mice treated with Isomer B both exhibited some progression in the symptoms typical of Huntington's disease during the first three days following administration, the mice treated with Isomer B exhibited significantly less deterioration than the control mice during the period of 17 to 24 days after administration. In particular, deterioration in gait was substantially arrested or slowed during this period, and the incidences of involuntary movements such as involuntary chorea, tremors and twitches in the Isomer B-treated mice were no worse after 21 days than they had been prior to

administration of the Isomer B. It is conceivable that by repeating the administration of Isomer B at appropriate intervals (which was not done in the tests), the development of the symptoms could be arrested or slowed still further.

Thus, the results indicate that Isomer B would be useful in preventing the onset of, or slowing the development of, the symptoms associated with Huntington's disease.

EXAMPLE 6

Comparison of the Sedative Properties of Tetrabenazine and the Dihydrotetrabenazine Isomers B and C

A study was carried out in rats to determine whether the dihydrotetrabenazine isomers of the invention have sedative properties. The effects of the isomers on spontaneous locomotor activity in rats were compared with the effects produced by tetrabenazine and haloperidol using the methods set out below. The results are shown in Table 10.

Methods

Male Sprague-Dawley rats, (Charles River Laboratories, Saint-Germain/L'Arbresle, France), weighing 200-250 g at the beginning of the study, were used for the studies. The rats were housed, 2 or 3 per cage, in Makrolon type III cages, in a room set up with the following environmental conditions: temperature: 20 ± 2 °C, humidity : minimum 45 %, air changes: > 12 per hour, light/dark cycle of 12 h/12 h [on at 7:00 a.m.]. The rats were allowed to acclimatize to their conditions for at least five days before commencement of the study. The rats received food (Dietex, Vigny, France, ref. 811002) and water (tap water in water bottle) *ad libitum*.

Solutions of each test compound in corn oil were freshly prepared on the day of the experiment. Haloperidol was prepared in hydroxyethylcellulose, 0.5% in deionized water. Either the vehicle or the test compounds were administered as a single dose (0.3, 1, 3 and 10 mg/kg, 2 mL/kg i.p.). The reference compound haloperidol (1 mg/kg) was administered i.p. (2 mL/kg).

The animals were placed in plexiglass cages under a video camera in a room with low light intensity (maximum 50 lux). At forty five minutes and 3 hours after

administration, locomotor activity was determined during 20 minute periods using a video image analyzer (Videotrack, View Point, France). Locomotor activity was recorded in the reference group (haloperidol) at 1 hour after administration. The number and duration of ambulatory movements and duration of inactivity was measured. At the end of the locomotor activity measurement (45 minutes and 3 hours), palpebral closure and arousal were be scored as follows in the plexiglass cage :

Palpebral closure :

0 : (normal) eyelids wide open
1 : eyelids slightly drooping
2 : ptosis, drooping eyelids approximately half-way
3 : eyelids completely shut

Arousal :

1 : very low, stupor, coma, little or no responsiveness
2 : low, some stupor, « dulled », some head or body movement
3 : somewhat low, slight stupor, some exploratory movements with periods of immobility
4 : normal, alert, exploratory movements /slow freeze
5 : somewhat high, slight excitement, tense, sudden darting or freezing
6 : very high, hyper alert, excited, sudden bouts of running or body movements

The number of occurrences and duration (in seconds) of ambulatory (large) movements and the duration of periods of inactivity (seconds) was determined during two 20 minute periods (45 minutes and 3 hours after administration) using a video image analyzer (Videotrack, ViewPoint, Lyon, France). Image tracking was performed using a video camera placed above the plexiglass cage, recording overall locomotor activity. Images recorded with the video camera were digitalized and displacement of the centre of gravity of the digital image spots was tracked and analyzed using the following method: the speed of displacement of the centre of gravity of the spot was measured and two threshold values were set to define the type of movement: threshold 1 (high speed) and threshold 2 (low speed). When the

animal moved and the speed of displacement of the centre of gravity of the spot was above threshold 1, the movement was considered as an ambulatory movement. When the animal remained inactive, the speed was below threshold 2, the movement was considered as inactivity.

The results were expressed as the means ± SEMs of the 12 individual values. Statistical analyses were carried out using ANOVA (one way) and Dunnett's t-test and with the non parametric test of Kruskal-Wallis followed by a Mann & Whitney U-test for the sedation cotation. A p value of $p<0.05$ was taken as indicating significance.

Protocol

Group size n=12
Group 1: Reference, haloperidol (1 mg/kg i.p.)
Group 2: Vehicle control group (2 ml/kg i.p.)
Group 3: tetrabenazine (0.3 mg/kg i.p)
Group 4: tetrabenazine (1 mg/kg i.p)
Group 5: tetrabenazine (3 mg/kg i.p)
Group 6: tetrabenazine (10 mg/kg i.p)
Group 7: Isomer C (0.3 mg/kg i.p)
Group 8: Isomer C (1 mg/kg i.p)
Group 9: Isomer C (3 mg/kg i.p)
Group 10: Isomer C (10 mg/kg i.p)
Group 11: Isomer B (0.3 mg/kg i.p)
Group 12: Isomer B (1 mg/kg i.p)
Group 13: Isomer B (3 mg/kg i.p)
Group 14: Isomer B (10 mg/kg i.p)

Results

Table 10
Effects of Tetrabenazine, Isomer B, Isomer C (0.3, 1, 3 and 10 mg/kg i.p.) on spontaneous locomotor activity in rats

Observation time : 45 minutes after administration				
		Large movements		**Inactivity**
Treatment	**Dose (mg/kg)**	**Occurrence**	**Duration (sec)**	**Duration (sec)**
Vehicle	2 mL/kg	286 ± 35	76.4 ± 10.9	349.0 ± 37.4
Haloperidol	1 mg/kg	58 ± 33 **	14.8 ± 8.5 **	637.2 ± 60.1 **
Tetrabenazine	0.3 mg/kg	253 ± 32	66.8 ± 10.7	390.4 ± 37.4
Tetrabenazine	1 mg/kg	189 ± 32	46.5 ± 8.6	456.5 ± 50.5
Tetrabenazine	3 mg/kg	38 ± 25 **	8.7 ± 5.9 **	697.8 ± 39.7 **
Tetrabenazine	10 mg/kg	1 ± 1 **	0.2 ± 0.2 **	723.1 ± 46.5 **
Isomer C	0.3 mg/kg	285 ± 34	79.2 ± 10.0	323.7 ± 25.6
Isomer C	1 mg/kg	295 ± 30	71.8 ± 8.3	324.6 ± 38.1
Isomer C	3 mg/kg	308 ± 36	84.0 ± 9.4	322.7 ± 27.8
Isomer C	10 mg/kg	254 ± 32	66.5 ± 9.9	368.7 ± 30.9
Isomer B	0.3 mg/kg	268 ± 36	72.0 ± 9.6	346.1 ± 36.9
Isomer B	1 mg/kg	297 ± 22	87.0 ± 7.6	334.0 ± 23.2
Isomer B	3 mg/kg	313 ± 38	89.1 ± 12.4	342.2 ± 33.3
Isomer B	10 mg/kg	298 ± 37	84.0 ± 11.2	333.1 ± 26.9
Observation time : 3 hours after administration				
		Large movements		**Inactivity**
Treatment	Dose (mg/kg)	Occurrence	Duration (sec)	Duration (sec)
Vehicle	2 mL/kg	101 ± 23	24.8 ± 6.0	540.9 ± 37.5
Haloperidol	1 mg/kg	9 ± 8 **	2.2 ± 2.0 **	723.6 ± 50.2 **
Tetrabenazine	0.3 mg/kg	96 ± 14	24.3 ± 4.2	545.9 ± 37.1
Tetrabenazine	1 mg/kg	90 ± 16	21.5 ± 4.0	556.9 ± 31.1

Tetrabenazine	3 mg/kg	9 ± 4 **	1.7 ± 0.9 **	729.9 ± 26.8 **
Tetrabenazine	10 mg/kg	3 ± 1 **	0.6 ± 0.3 **	762.1 ± 40.7 **
Isomer C	0.3 mg/kg	113 ± 19	31.4 ± 6.0	519.3 ± 33.7
Isomer C	1 mg/kg	128 ± 24	30.3 ± 6.5	510.2 ± 44.9
Isomer C	3 mg/kg	125 ± 22	30.2 ± 5.5	493.6 ± 38.5
Isomer C	10 mg/kg	164 ± 30	42.7 ± 8.0	465.7 ± 49.0
Isomer B	0.3 mg/kg	101 ± 29	28.9 ± 9.2	566.4 ± 44.3
Isomer B	1 mg/kg	125 ± 18	34.5 ± 6.2	525.8 ± 28.6
Isomer B	3 mg/kg	113 ± 17	31.1 ± 6.5	530.5 ± 38.0
Isomer B	10 mg/kg	120 ± 26	30.9 ± 6.4	515.0 ± 53.0

**Significantly different from Vehicle group (p,0.01)ANOVA one way followed by Dunnett's test.

The results demonstrate that tetrabenazine produces a dose-dependent sedative effect 45 minutes and 3 hours after administration whereas Isomer B and Isomer C show no sedative effects at any time, although isomer C does show a slight and non-significant hyperlocomotor effect 3 hours after administration. .

EXAMPLE 7

Pharmaceutical Compositions

(i) Tablet Formulation -I

A tablet composition containing a dihydrotetrabenazine of the invention is prepared by mixing 50mg of the dihydrotetrabenazine with 197mg of lactose (BP) as diluent, and 3mg magnesium stearate as a lubricant and compressing to form a tablet in known manner.-

(ii) Tablet Formulation - II

A tablet composition containing a dihydrotetrabenazine of the invention is prepared by mixing the compound (25 mg) with iron oxide, lactose, magnesium stearate, starch maize white and talc, and compressing to form a tablet in known manner.

(iii) Capsule Formulation

A capsule formulation is prepared by mixing 100mg of a dihydrotetrabenazine of the invention with 100mg lactose and filling the resulting mixture into standard opaque hard gelatin capsules.

Equivalents

It will readily be apparent that numerous modifications and alterations may be made to the specific embodiments of the invention described above without departing from the principles underlying the invention. All such modifications and alterations are intended to be embraced by this application.

CLAIMS

1. 3,11b-*cis*-dihydrotetrabenazine for use in halting or slowing the progress of one or more symptoms of Huntington's disease in a patient, and more particularly a symptom selected from involuntary movements such as involuntary chorea, tremors and twitches, and degeneration in gait.

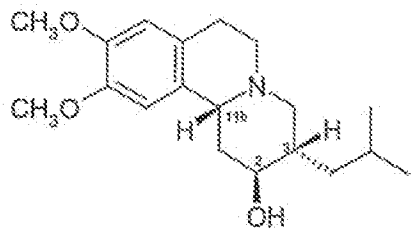
2. 3,11b-*cis*-dihydrotetrabenazine for use in the prophylactic treatment of a patient identified as carrying the mutant gene responsible for Huntington's disease.

3. 3,11b-*cis*-dihydrotetrabenazine for use in the prophylactic treatment of a patient within the age range 15 – 50 years who is carrying the mutant form of the HD gene but who have not yet developed symptoms of the disease, the prophylactic treatment being for the purpose of preventing or slowing the onset of symptoms associated with Huntington's disease.

4. 3,11b-*cis*-dihydrotetrabenazine for use according to any one of claims 1 to 3 wherein the 3,11b-*cis*-dihydrotetrabenazine in substantially pure form, for example at an isomeric purity of greater than 90%, typically greater than 95% and more preferably greater than 98%.

5. 3,11b-*cis*-dihydrotetrabenazine for use according to any one of the preceding claims wherein the 3,11b-*cis*-dihydrotetrabenazine is in a (+)-isomeric form.

6. 3,11b-*cis*-dihydrotetrabenazine for use according to any one of the preceding claims wherein the 3,11b-*cis*-dihydrotetrabenazine has the formula (Ia):



(Ia)

7. 3,11b-*cis*-dihydrotetrabenazine for use according to any one of the preceding claims wherein the dihydrotetrabenazine is in the form of an acid addition salt.

8. 3,11b-*cis*-dihydrotetrabenazine for use according to claim 7 wherein the salt is a methane sulphonate salt.

9. The use of 3, 11b-*cis*-dihydrotetrabenazine as defined in any one of the preceding claims for the manufacture of a medicament for halting or slowing the progress of one or more symptoms of Huntington's disease, and more particularly a symptom selected from involuntary movements such as involuntary chorea, tremors and twitches, and gait degeneration.

10. The use of 3, 11b-*cis*-dihydrotetrabenazine as defined in any one of the preceding claims for the manufacture of a medicament for the prophylactic treatment of a patient identified as carrying the mutant gene responsible for Huntington's disease.

11. The use of 3,11b-*cis*-dihydrotetrabenazine as defined in any one of the preceding claims for the manufacture of a medicament for the prophylactic treatment of a patient within the age range 15 – 50 years who is carrying the mutant form of the HD gene but who has not yet developed symptoms of the disease, the prophylactic treatment being for the purpose of preventing or slowing the onset of symptoms associated with Huntington's disease.

12. A method of halting or slowing the progress of one or more symptoms of Huntington's disease, and more particularly a symptom selected from involuntary movements such as involuntary chorea, tremors and twitches, and gait degeneration, in a patient in need of such treatment, which method comprises the administration of an effective therapeutic amount of 3,11b-*cis*-dihydrotetrabenazine as defined in any one of the preceding claims.

13. A method for the prophylactic treatment of a patient identified as carrying the mutant gene responsible for Huntington's disease, the method comprising administering to the patient a *cis*-dihydrotetrabenazine as

defined in any one of the preceding claims in an amount effective to prevent or slow down the onset or progression of the disease.

14. A compound for use, method or use according to any one of the preceding claims wherein a patient to whom the *cis*-dihydrotetrabenazine is administered carries a mutant form of the gene in which the number of CAG repeats on the IT-15 gene is at least thirty five, more typically at least forty, for example at least 45, or at least 50.

15. A method for the prophylactic treatment of a patient identified as carrying the mutant gene responsible for Huntington's disease, the method comprising administering to the patient a *cis*-dihydrotetrabenazine as defined in any one of the preceding claims in an amount effective to prevent or slow down sub-clinical progression of the disease.

16. A compound for use, method or use substantially as described herein with reference to the examples.

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2006/002593

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/473 A61P25/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, EMBASE, BIOSIS, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 2005/077946 A (CAMBRIDGE LAB LTD [GB]; TRIDGETT ROBERT [GB]; CLARKE IAN [GB]; TURTLE) 25 August 2005 (2005-08-25) page 5, line 20 - page 10, line 2 claims 1-34	1-16
X,P	WO 2006/053067 A2 (PRESTWICK PHARMACEUTICALS INC [US]; CLARENCE-SMITH KATHLEEN [US]) 18 May 2006 (2006-05-18) page 4, lines 5-20 page 5, lines 15-28	1-16

-/--

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 November 2006	29/11/2006

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Young, Astrid

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2006/002593

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KILBOURN M R ET AL: "Absolute Configuration of (+)-alpha-Dihydrotetrabenazine, an Active Metabolite of Tetrabenazine" CHIRALITY, WILEY-LISS, NEW YORK, US, vol. 9, no. 1, 1997, pages 59-62, XP002329921 ISSN: 0899-0042 the whole document	1-16
A	KILBOURN M ET AL: "Binding of alpha-dihydrotetrabenazine to the vesicular monoamine transporter is stereospecific" EUROPEAN JOURNAL OF PHARMACOLOGY, AMSTERDAM, NL, vol. 278, no. 3, 1995, pages 249-252, XP002329922 ISSN: 0014-2999 the whole document	1-16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2006/002593

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 12-16 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2006/002593

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005077946 A	25-08-2005	AU 2005213525 A1	25-08-2005
		CA 2555815 A1	25-08-2005
		EP 1716145 A1	02-11-2006
WO 2006053067 A2	18-05-2006	NONE	